

10/pets
10/552298

PHOSPHATASE REGULATION OF NUCLEIC ACID
TRANSCRIPTION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Serial No. 60/459,786, filed April 1, 2003, which is incorporated herein by reference.

STATEMENTS REGARDING FEDERALLY SPONSORED RESEARCH

[0002] The invention was funded in part by Grant No. 2RDK13149 awarded by the National Institutes of Health and by Grant No. GM33300 awarded by the National Institutes of Health. The government may have certain rights in the invention.

TECHNICAL FIELD

[0003] This invention relates generally to the regulation of transcription, and more specifically to the regulation of neuronal gene expression.

BACKGROUND

[0004] Protein phosphatases are enzymes that reverse the actions of protein kinases by cleaving phosphate from serine, threonine, and/or tyrosine residues in proteins. Serine/threonine protein phosphatases are associated with the regulation of cellular gene expression, which occurs primarily at the level of transcription initiation by RNA polymerase. Regulated transcription initiation by RNA polymerase (RNAP) II in higher eukaryotes involves the formation of a complex with general transcription factors at promoters. The largest subunit of RNAP II contains a C-terminal domain (CTD) comprised of multiple repeats of the

consensus sequence Tyr¹Ser²Pro³Thr⁴Ser⁵Pro⁶Ser⁷. The progression of RNAP II through the transcription cycle is regulated by both the state of CTD phosphorylation and the specific site of phosphorylation within the consensus repeat.

[0005] Specific kinases catalyze phosphorylation of Ser 2 and of Ser 5 in the multiple heptad repeats in the CTD of RNAP II. Unphosphorylated RNAP II (RNAP IIA) enters the pre-initiation complex where TFIIH catalyzes phosphorylation of Ser 5 to enhance the 7-methyl G capping reaction. PTEFb catalyzes phosphorylation of Ser 2, a process necessary for transcript elongation. Phospho RNAP II (RNAP IIO) is ultimately dephosphorylated by FCP1 allowing recycling of the enzyme and re-initiation of transcription.

[0006] Mechanisms that regulate the phosphorylation and dephosphorylation of transcription-associated factors can effectively repress and activate transcription of particular genes during. Such mechanisms are particularly important in cellular development and differentiation. Identifying the factors involved in gene activation and repression provides an opportunity to control the differentiation of, for example, stem cells in to specialized cell types.

Summary

[0007] Novel nucleic acid sequences encoding novel small CTD phosphatase (SCP) polypeptides, and dominant-negative mutants thereof, are disclosed. In addition, methods related to identifying substances that modify gene transcription, methods of modifying stem cell differentiation, and methods of treating disease conditions resulting from insufficient, increased or aberrant

production of SCP polypeptides are provided. These methods include the use of substances that bind to, or interact with, the SCP proteins, (naturally occurring and biologically active, also referred to herein as wild type SCP proteins) genes encoding the SCP proteins, SCP messenger RNA, or the use of genetically altered SCP proteins.

[0008] In one embodiment, isolated nucleic acid molecules are provided. Such nucleic acid molecules include those; 1) consisting of a nucleotide sequence which is at least 80% identical to the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11; 2) comprising a nucleotide sequence which is at least 80%, 90% or 95% identical to the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11; 3) encoding a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12; 4) encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12; 5) encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12 with 0 to 50, 0 to 30, or 0 to 10 conservative amino acid substitutions; and 6) encoding a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12, such that the nucleic acid molecule hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 1, 3, 5, 7, 9 or 11, or a complement thereof, under stringent conditions.

[0009] In another embodiment, isolated nucleic acid molecules deposited as ATCC Accession Numbers BE300370, AL520011, and AL520463, or a complement thereof, are provided.

[0010] In other embodiments nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, 3, 5 ,

7, 9 or 11 or consisting of the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11, are provided.

[0011] Also provided are vectors containing the nucleic acid molecules disclosed herein and host cells containing such vectors. Methods of producing a polypeptide by culturing such host cells are also provided.

[0012] In yet another embodiment, isolated polypeptides are provided. Such polypeptides include a polypeptide: 1) consisting of an amino acid sequence which is at least 80%, 90% or 95% identical to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12; 2) comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 1; 3) comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12 with 0 to 50, 0 to 30 or 0 to 10 conservative amino acid substitutions; 4) encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 80%, 90% or 95% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11; and 5) that are naturally occurring allelic variants of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6 or 8, such that the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 1, 3, 5, 7, 9 or 11, or a complement thereof, under stringent conditions.

[0013] Also included are polypeptides comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12 and polypeptides consisting of the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12. Such polypeptides are generally phosphatases or a phosphatase inactive mutant. The phosphatase is generally a serine phosphatase that dephosphorylates serine 5 within the C-terminal binding

domain (CTD) of RNA polymerase II. The phosphatase can be small CTD phosphatase-1 (SCP1), small CTD phosphatase-2 (SCP2), or small CTD phosphatase-3 (SCP3).

[0014] Also provided are antibodies that selectively bind to a polypeptides provided herein.

[0015] In another embodiment, methods of promoting differentiation of a non-neuronal cell in to a cell of the nervous system are provided. Such methods include contacting the non-neuronal cell with a nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:12 and expressing the polypeptide in the cell such that the dominant-negative SCP mutant inhibits the activity of endogenous SCP. Such methods are useful for promoting, for example, differentiation of stem cells in to nerve tissue including, but not limited to, neurons, sensory neurons, motoneurons, interneurons, glial cells, microglial cells and astrocytes.

[0016] In another embodiment, a method of inhibiting differentiation of a non-neuronal cell in to a cell of the nervous system by contacting the cell with a nucleic acid molecule including a nucleic acid sequence encoding a polypeptide selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8, and expressing the polypeptide in the cell, is provided.

[0017] Also provided are methods of promoting RNA polymerase II associated transcription in a cell by contacting the cell with a nucleic acid molecule including a nucleic acid sequence encoding a polypeptide selected from SEQ ID NO:10 and SEQ ID NO:12, and expressing the polypeptide in the cell.

[0018] In another embodiment, a composition that includes an inhibitor of small CTD phosphatase (SCP) gene expression is provided. The inhibitor can be a small molecule inhibitor of gene expression, an anti-sense oligonucleotide, or a small interfering RNA molecule (siRNA or RNAi). The inhibitor of SCP gene expression can, for example, specifically bind to a polynucleotide that includes: 1) a sequence selected from the group consisting of SEQ ID NO:1, 3, 5 and 7; 2) a complement of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, 5 and 7; 3) a reverse sequence of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, 5 and 7; 4) a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, 4, 6 and 8; 5) a complement of a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, 4, 6 and 8; or 6) a reverse sequence of a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:2, 4, 6 and 8.

[0019] In another embodiment, a method of promoting the differentiation of a non-neuronal cell into a cell of the nervous system is provided. Such methods can be accomplished by contacting the non-neuronal cell with the composition described above in a sufficient concentration to inhibit the expression of a small CTD phosphatase (SCP).

[0020] In yet another embodiment, a method for identifying a compound that modulates the activity of an SCP polypeptide is provided. The method includes contacting an SCP polypeptide provided herein with a test compound and determining the effect of the test compound on the activity

of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

[0021] In another embodiment, a method of modulating the differentiation of a mammalian stem cell by contacting the stem cell with a compound that modulates SCP1, SCP2 or SCP3 activity, under conditions suitable for differentiation of said stem cell, is provided.

[0022] In another embodiment, a method of transplanting a mammalian stem cell or progenitor cell to a patient in need thereof including (a) contacting the stem cell or progenitor cell with a compound that inhibits SCP1, SCP2 or SCP3 activity to produce a treated stem cell or progenitor cell; and (b) transplanting the treated stem cell into said patient, is provided.

Brief Description of the Drawings

[0023] Figure 1A depicts a sequence alignment of amino acid sequences surrounding the catalytic domain and relation of SCP to FCP1.

[0024] Figure 1B depicts the domain structures of FCP1 and SCP polypeptides.

[0025] Figure 2A is a graph depicting phosphatase activity of SCP1 at various pH.

[0026] Figure 2B is a graph depicting the divalent metal ion requirements for SCP1 phosphatase activity.

[0027] Figure 2C is an autoradiogram depicting a CTD phosphatase assay of FCP1 on GST-CTDo and RNAP IIO prepared by MAPK2/ERK2.

[0028] Figure 2D is an autoradiogram depicting a CTD phosphatase assay of SCP1 on GST-CTDo and RNAP IIO prepared by MAPK2/ERK2.

[0029] Figure 3A is an autoradiogram and graph depicting substrate specificity of SCP1 for dephosphorylation of RNAP IIO prepared with various CTD kinases.

[0030] Figure 3B is a graph depicting the effects of GST-SCP1 214 on a 28 aa peptide consisting of heptad repeats containing either Ser 5 phosphate or Ser 2 phosphate.

[0031] Figure 4 is an autoradiogram depicting the effect of RAP74 on CTD phosphatase activity of SCP1 and SCP2.

[0032] Figure 5A depicts nuclear localization and association of SCP1 with RNAP II. Cells were co-stained for the endosomal marker EEA1 using mouse anti-EEA1 and Alexa Fluor 594 conjugated goat anti-mouse (red). Nuclei were detected with DAPI (blue).

[0033] Figure 5B depicts immunofluorescence microscopy detection of endogenous SCP1 using rabbit polyclonal IgG 6307 and Alexa Fluor 488 conjugated goat anti-rabbit IgG (green).

[0034] Figure 5C is a gel depicting co-immunoprecipitation of RNAP II and endogenous SCP1.

[0035] Figure 6A is a graph depicting the effects of targeted SCP1 261 on reporter gene expression.

[0036] Figure 6B is a graph depicting the effects of SCP1 261 and mutant SCP1 261 on basal promoter activity.

[0037] Figure 6C is a graph depicting the differing effects of SCP1 261 and phosphatase-inactive SCP1 261 on Gal 4-VP16 stimulated gene expression.

[0038] Figure 6D is a graph depicting the effects of SCP1 261 and phosphatase-inactive SCP1 261 on ligand activated receptor activity.

[0039] Figure 6E is a graph depicting competitive effects of mutant SCP1 261 with SCP1 261.

[0040] Figure 7A depicts Northern blot analysis of the expression of SCP1 in human tissues.

[0041] Figure 7B depicts in situ hybridization analysis of expression of SCP1 in e 10.5 mouse cervical spinal cord.

[0042] Figure 7C depicts in situ analysis of the expression of isl-1 in areas of the developing spinal cord where SCP1 is not expressed.

[0043] Figure 8A depicts co-immunoprecipitation of SCP1 and REST/NRSF.

[0044] Figure 8B depicts chromatin immunoprecipitation using anti-SCP antibody.

[0045] Figure 9A depicts undifferentiated P19 cells.

[0046] Figure 9B depicts P19 cells differentiated into neuron like cells (NLC) by treatment with retinoic acid and growth in selective medium.

[0047] Figure 9C depicts differentiated GFP expressing P19 cells.

[0048] Figure 9D depicts differentiated mutant SCP1-expressing P19 cells.

[0049] Figure 9E depicts differentiated SCP1-expressing P10 cells.

[0050] Figure 9F depicts differentiated REST/NRSF-expressing P19 cells.

[0051] Figure 10 depicts the quantitation of transcripts using real time quantitative RT-PCR and the effect of siRNA on the transcript quantity.

Detailed Description

[0052] Novel small CTD phosphatases (SCP's) polypeptides, and nucleic acid molecules encoding such polypeptides, are provide. Also provided are methods of modifying gene transcription by modulating the expression and/or activity

of SCP's. Such methods can be used to inhibit or promote differentiation of cells. Also provided are methods for identifying nucleic acids compounds that bind to, or interact with, one or more SCP proteins or the DNA/RNA encoding the SCP proteins and, thus, modifying the activity of an SCP protein on RNA polymerase II, or on other transcription factors essential to gene transcription.

[0053] Compounds that bind to, or interact with, one, or more SCP proteins or the DNA/RNA encoding the proteins can inhibit or enhance the activity of the RNA polymerase II, thus, inhibiting or enhancing gene transcription. For example, antisense, nonsense or interfering (i.e., RNAi) nucleotide sequences that modulate SCP translation or transcription can effect the RNA polymerase II-mediated gene transcription.

[0054] Unphosphorylated RNAP II, designated RNAP IIA, enters the pre-initiation complex where phosphorylation of Ser 5 is catalyzed by TFIIH (which contains cdk7/cyclin H subunits) concomitant with transcript initiation. This generates the phosphorylated form of RNAP II, designated RNAP IIO. Ser 5 phosphorylation facilitates the recruitment of the 7-methyl G capping enzyme complex (Cho et al., (1997) Genes Dev. 11:3319-3326; McCracken et al., (1997) Genes Dev. 11:3306-3318). Phosphorylation of Ser 2, is catalyzed by the cyclin-dependent kinase P-TEFb (which contains cdk9/cyclin T subunits). During transcript elongation in yeast there is extensive turnover of Ser 2 phosphates mediated by FCP1 and Ctk1, the putative PTEFb homolog (Cho et al., (2001) Genes Dev. 15:3319-3329). Finally, dephosphorylation of Ser 2 by the FCP1 phosphatase regenerates RNAP IIA thereby completing the cycle.

[0055] FCP1 is a class C (PPM) phosphatase containing a BRCT domain that is required for interaction with RNAP II and dephosphorylation of the CTD (Cho et al, (1999) Genes Dev. 13:1540-1552; Archambault et al., (1997) Proc. Natl. Acad. Sci. USA 94:14300-14305). FCP1 interacts with and is stimulated by RAP74, the larger subunit of TFIIF. Class C phosphatases are resistant to inhibitors that block other classes of Ser/Thr phosphatases and bind Mg²⁺ or Mn²⁺ in the binuclear metal center of the catalytic site. The $\Psi\Psi\Psi\text{DXDX}(\text{T/V})\Psi\Psi$ motif (where Ψ =hydrophobic residue) present in the FCP1 homology domain characterizes a subfamily of class C phosphatases with both Asp residues being essential for activity.

[0056] Synthetic lethality is observed between mutant FCP1 and reduced levels of RNAP II in *S.cerevisiae* and *S. pombe*, indicating that FCP1 is an essential gene (Kobor et al., (1999) Mol. Cell 4:55-62; Kimura et al., (2002) Mol. Cell Biol. 22:1577-1588). Mammalian FCP1 dephosphorylates both Ser 2 and Ser 5 in vitro in the context of native RNAP II.

[0057] Although FCP1 is the only reported CTD phosphatase, examination of the databases reveals additional genes that consist principally of a domain with homology to the CTD phosphatase domain of FCP1. Three closely related human genes encoding small proteins with CTD phosphatase domain homology, but lacking a BRCT domain, have been identified. In the present study we show that a gene located on chromosome 2 encodes a nuclear CTD phosphatase. This protein preferentially dephosphorylates Ser 5 within the CTD of RNAP II and is stimulated by RAP74. Expression of this small CTD phosphatase (SCP1) inhibits activated transcription from a variety of promoter-reporter gene constructs whereas expression of a mutant lacking

phosphatase activity enhances transcription. These novel small CTD phosphatase are involved in the regulation of RNAP II transcription.

[0058] The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as SCP protein and nucleic acid molecules, which comprise a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics associated with serine phosphatase activity, and particularly with dephosphorylation of the CTD of RNA Polymerase II.

[0059] In the vertebrate spinal cord expression of a temporally ordered sequence of transcriptional repressors and activators direct formation of glia and specialized neuronal cell types from common precursors (Jessell, Nature Rev Genetics (2000) 1:20-29.). Repressor systems also direct long term silencing of inappropriate gene expression in differentiated cells. A 23 bp DNA element (repressor element 1 (RE-1)) binds the Zn²⁺ - finger-containing protein REST (RE-1 silencing transcription factor)/NRSF (Neuron-Restrictive Silencer Factor) (Chong, et al., Cell (1995) 80:949-957; Schoenherr and Anderson.

Science (1995) 267:1360-1363; Chen, et al., Nature Genetics (1998) 20:136-142.). REST/NRSF recruits a multiprotein complex that acts to repress gene transcription via histone deacetylation and via methylation of DNA and of histone H3 (Naruse, et al. Proc. Natl. Acad. Sci USA (1999) 96:1369-13696; Hakimi, et al. Proc. Natl. Acad. Sci. USA (2002) 99:7420-7425; Kokwra, et al. J. Biol. Chem. (2001) 276:34115-34121). These defined mechanisms of gene silencing via REST/NRSF result from covalent modifications of chromatin.

[0060] The present studies further identify SCPs as functional components of the REST/NRSF silencing complex. Because phosphorylation of serine 5 of the CTD of RNAP II is essential for initiation of transcription, dephosphorylation is an effective and reversible mechanism to inhibit transcription. SCPs repress transcription of a variety of regulated reporter genes but in vivo are specifically recruited to RE-1 elements in neuronal genes. Other genes that contain an FCP1 phosphatase homology domain defined by the YYY DX(T/V) YY (where Y represents a hydrophobic residue) sequence (Kang and Dahmus, (1993) J. Biol. Chem. 268:25033-25040; Chambers and Dahmus, (1994) J. Biol. Chem. 269:26243-26248) are candidates to function in regulating other classes of genes.

[0061] As P19 stem cells differentiate into neurons both SCPs and REST/NRSF expression are silenced indicating that silencers of neuronal silencers (SONS) exist. Inhibition of SCP by a dominant negative form of SCP in replicating P19 cells increases the fraction of stem cells that morphologically develop into neurons indicating that the same mechanisms that silence neuronal gene expression in

differentiated non-neuronal cells act in neuronal stem cells.

[0062] In addition, an siRNA-mediated decrease in the *Drosophila* SCP gene product on neuronal gene expression in S2 cells was also identified, further indicating that SCP is part of REST/NRSF complexes and functions in silencing neuronal gene expression. Dephosphorylation of Ser 5 of the CTD of RNAP II is a target for reversible mechanisms involved in the inhibition of neuronal gene transcription using, for example, siRNA.

Nucleic acids and Polypeptides

[0063] The SCP proteins, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2, 4, 6, 8, 10 or 12, thereof are collectively referred to as "polypeptides or proteins of the invention" or SCP "polypeptides or proteins". Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "SCP nucleic acids." SCP molecules refer to SCP nucleic acids, polypeptides, and antibodies.

[0064] As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a cDNA or genomic DNA) and RNA molecules (e.g., an mRNA) and analogs of the DNA or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

[0065] The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated"

includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

[0066] As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred, example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are

hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. For example, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 5 or 7 corresponds to a naturally occurring nucleic acid molecule.

[0067] The definitions of the terms "complement", "reverse complement" and "reverse sequence", as used herein, are best illustrated by the following example. For the sequence 5' AGGACC 3', the complement, reverse complement and reverse sequence are as follows:

[0068] complement: 3' TCCTGG 5'

[0069] reverse complement: 3' GGTCCT 5'

[0070] reverse sequence: 5' CCAGGA 3'.

[0071] Preferably, sequences that are complements of a specifically recited polynucleotide sequence are complementary over the entire length of the specific polynucleotide sequence.

[0072] As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

[0073] As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open

reading frame encoding a SCP protein, preferably a mammalian SCP protein, and can further include non-coding regulatory sequences, and introns.

[0074] An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of SCP protein having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-SCP protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-SCP chemicals. When the SCP protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

[0075] A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of SCP1, SCP2 or SCP3 (e.g., the sequence of SEQ ID NO:1, 3, or 5 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers BE300370, AL520011, or AL520463,) without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present

invention are predicted to be particularly unamenable to alteration.

[0076] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a SCP protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a SCP coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for SCP biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or 8, the resulting dominant-negative mutants have a sequence set forth in SEQ ID NO:10 or 12, respectively.

[0077] As used herein, a "biologically active portion" of a SCP protein includes a fragment of a SCP protein which participates in an interaction between a SCP molecule and a non-SCP molecule, e.g. RNA polymerase II or REST/NRSF. Biologically active portions of a SCP protein include peptides comprising amino acid sequences sufficiently

homologous to or derived from the amino acid sequence of the SCP protein, e.g., the amino acid sequence shown in SEQ ID NO:2, 4 or 6, which include less amino acids than the full length SCP proteins, and exhibit at least one activity of a SCP protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the SCP protein, e.g., dephosphorylation of RNA polymerase II or interacting with REST/NRSF. A biologically active portion of a SCP protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a SCP protein can be used as targets for developing agents which modulate a SCP mediated activity, e.g., the regulation of differentiation of a non-neuronal cell into a neuronal cell.

[0078] Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

[0079] To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., SEQ ID NO:2, 4 or 6). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared.

When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0080] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (J. Mol. Biol. (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available on the world wide web at [gcg.com](http://www.gcg.com)), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6.

[0081] The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0082] The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to SCP nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to SCP protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See the world wide web at ncbi.nlm.nih.gov.

Isolated Nucleic Acid Molecules

[0083] In one aspect, the invention provides, an isolated or purified, nucleic acid molecules that encode SCP polypeptides described herein, e.g., a full length SCP1, SCP2, SCP3 or SCP1 214 protein or a fragment thereof, e.g., a biologically active portion of an SCP protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to identify nucleic acid molecule encoding a polypeptide of the invention, SCP mRNA, and fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

[0084] In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1, 3, 5 or 7, or mutant derivatives thereof including SEQ ID NO:9 or 11. In one embodiment, the nucleic acid molecule includes sequences encoding the human SCP1, SCP2, or SCP3 protein (i.e., "the coding region", from nucleotides 1-781 SEQ ID NO:1; nucleotides 1-852 SEQ ID NO:3; nucleotides 1-798 SEQ ID NO:3), as well as 5' untranslated sequences. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to the mature protein of SEQ ID NO:2, 4, 6 or 8.

[0085] In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, or 7, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5 or 7 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 3, 5 or 7, thereby forming a stable duplex.

[0086] In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 3, 5 or 7, or a portion, preferably of the same length, of any of these nucleotide sequences.

SCP Nucleic Acid Variants

[0087] The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 5 or 7, or the dominant negative SCP mutants provided in SEQ ID NO:9 and 11. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same SCP proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2, 4, 6, or 8. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

[0088] Nucleic acids of the invention can be chosen for having codons, which are preferred, or non-preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in e. coli, yeast, human, insect, or CHO cells.

[0089] Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the

coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

[0090] In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO: 1, 3, 5 or 7, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology.

[0091] Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the nucleotide sequence shown in SEQ ID NO:2, 4, 6, or 8, or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:2, 4, 6, or 8 or a fragment of the sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the SCP cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the SCP gene.

[0092] Preferred variants include those that are correlated with dephosphorylation of RNA polymerase II, for example.

[0093] Allelic variants of SCP, e.g., human SCP, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the SCP protein within a population that maintain the ability to dephosphorylate RNA polymerase II. Functional allelic variants will typically contain

only conservative substitution of one or more amino acids of SEQ ID NO:2, 4, 6, or 8, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the SCP, e.g., human SCP, protein within a population that do not have the ability to dephosphorylate RNA polymerase II. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2, 4, 6 or 8, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

[0094] Moreover, nucleic acid molecules encoding other SCP family members and, thus, which have a nucleotide sequence which differs from the SCP sequences of SEQ ID NO:1, 3, 5 or 7, are intended to be within the scope of the invention.

Antisense Nucleic Acid Molecules, Ribozymes, Modified SCP Nucleic Acid Molecules and siRNA

[0095] In another embodiment, isolated SCP nucleic acid molecules which are antisense to SCP are provided. Such molecules can be used to inhibit the expression of SCP in a cell, for example a stem cell, such that the cell can differentiate in to a cell of the nervous system. As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be eukaryotic (e.g., a mammalian cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived

from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

[0096] "Cells of the nervous system" refers to cells that are specifically related to the nervous system of an animal. For example, a "cell of the nervous system" can be a "neuron" or a "nerve cell", which is an excitable cell specialized for the transmission of electrical signals over long distances. Neurons receive input from sensory cells or other neurons and send output to muscles or other neurons. Exemplary "neurons" include a "sensory neuron" that has sensory input, a "motoneuron" that has muscle outputs, or "interneuron" that connects only with other neurons. A "cell of the nervous system" can also be a specialized non-neuronal nervous cell, for example a glial cell, which is a cell that surrounds a neuron, providing mechanical and physical support and electrical insulation between neurons. Examples of glial cells include, but are not limited to, microglial cells and astrocytes.

[0097] An "antisense" nucleic acid can include a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire SCP coding strand, or to only a portion thereof (e.g., the coding region of human SCP1, SCP2, SCP3 or SCP1 215, corresponding to SEQ ID NO:1, 3, 5, and 7, respectively). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding SCP (e.g., the 5' and 3' untranslated regions).

[0098] An antisense nucleic acid can be designed such that it is complementary to the entire coding region of SCP

mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of SCP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of SCP mRNA, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

[0099] An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[00100] The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a SCP protein to thereby

inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[00101] In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier et al. (1987) Nucleic Acids. Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) FEBS Lett. 215:327-330).

[00102] In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. A ribozyme having specificity for a SCP-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a SCP cDNA disclosed herein (i.e., SEQ ID NO:1, 3, 5, or

7), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) Nature 334:585-591). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a SCP-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, SCP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) Science 261:1411-1418.

[00103] SCP gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the SCP (e.g., the SCP promoter and/or enhancers) to form triple helical structures that prevent transcription of the SCP gene in target cells. See generally, Helene, C. (1991) Anticancer Drug Des. 6(6):569-84; Helene, C. et al. (1992) Ann. N.Y. Acad. Sci. 660:27-36; and Maher, L.J. (1992) Bioassays 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

[00104] An SCP nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone

of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) Bioorganic & Medicinal Chemistry 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) supra; Perry-O'Keefe et al. Proc. Natl. Acad. Sci. 93: 14670-675.

[00105] PNAs of SCP nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of SCP nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) supra)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) supra; Perry-O'Keefe supra).

[00106] In another embodiment, isolated siRNA nucleic acid molecules which destabilize SCP transcripts are provided. Such molecules can be used to inhibit the expression of SCP in a cell, for example a stem cell, such that the cell can differentiate in to a cell of the nervous system.

[00107] The identification of potential siRNA target sites in an SCP RNA sequence can be identified using the information provided herein. For example, the inventors have utilized siRNA in a Drosophila system to inhibit the expression of Drosophila SCP (e.g., see Figure 10). This information, coupled with the knowledge of the skilled artisan, can be used to generate siRNA suitable for use in other cell types, such as mammalian stem cells.

[00108] To identify sites in a target transcript (i.e., SCP RNA), the sequence of an RNA target of interest is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siRNA targets having complementarily to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siRNA molecules targeting those sites as well. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be

chosen to screen siRNA molecules for efficacy, for example by using in vitro RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siRNA construct to be used. High throughput screening assays can be developed for screening siRNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

[00109] The following non-limiting steps can be used to carry out the selection of siRNAs targeting a given gene sequence or transcript.

[00110] The target sequence is parsed in silico into a list of all fragments or subsequences of a particular length contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.

[00111] In some instances the siRNAs correspond to more than one target sequence; such would be the case for example in targeting many different strains of a viral sequence, for targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can

identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siRNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

[00112] In some instances the siRNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siRNA targets a gene with a paralogous family member that is to remain untargeted. A subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

[00113] The ranked siRNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

[00114] The ranked siRNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

[00115] The ranked siRNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

[00116] The ranked siRNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3' end of the sequence, and/or AA on the 5' end of the sequence (to yield

3' UU on the antisense sequence). These sequences allow one to design siRNA molecules with terminal TT thymidine dinucleotides.

[00117] Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siRNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siRNA duplex. If terminal TT residues are desired for the sequence then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

[00118] The siRNA molecules are screened in an in vitro, cell culture or animal model system to identify the most active siRNA molecule or the most preferred target site within the target RNA sequence.

[00119] The siRNA molecules of the invention can be designed to inhibit SCP gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siRNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates used for SCP activity. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to

distinguish among the functions of gene family members. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping.

[00120] In another embodiment, the siRNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as SCP genes. As such, siRNA molecules targeting multiple SCP targets can provide increased therapeutic effect. In addition, siRNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in development, such as prenatal development, postnatal development and/or aging.

[00121] In one embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by an SCP gene; (b) synthesizing one or more sets of siRNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siRNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In another embodiment, the siRNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length.

In yet another embodiment, the siRNA molecules of (b) are of differing length, for example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length.

Isolated SCP Polypeptides

[00122] In another embodiment, an isolated SCP protein, or fragment, e.g., a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-SCP antibodies are provided. SCP protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

[00123] Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events. The polypeptide can be expressed in systems, e.g., cultured cells, which result in substantially the same postranslational modifications present when expressed the polypeptide is expressed in a native cell, or in systems which result in the alteration or omission of postranslational modifications, e.g., glycosylation or cleavage, present when expressed in a native cell.

[00124] In one aspect, an SCP polypeptide has one or more of the following characteristics:

[00125] (i) it has the ability to inhibit cellular differentiation in to neuronal tissue;

[00126] (ii) it has a molecular weight, amino acid composition or other physical characteristic of SEQ ID NO:2, 4, or 6;

[00127] (iii) it has an overall sequence similarity of at least 50%, preferably at least 60%, more preferably at

least 70, 80, 90, or 95%, with a polypeptide of SEQ ID NO:2, 4 or 6;

[00128] (iv) it can be found in non-neuronal cells;

[00129] (v) it can colocalize to the nucleus with REST/NRSF; and

[00130] (vi) it has the ability to dephosphorylate the CTD of RNA polymerase II.

[00131] In another embodiment the SCP protein, or fragment thereof, differs from the corresponding sequence in SEQ ID NO:2, 4, 6 or 8. In one embodiment it differs by at least one but by less than 50, 30, 15, 10 or 5 amino acid residues. In another aspect, it differs from the corresponding sequence in SEQ ID NO:2, 4, 6 or 8 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it (if this comparison requires alignment the sequences should be aligned for maximum homology).

[00132] In another embodiment, dominant negative mutant SCP polypeptides are provided. SEQ ID NO:10 and 12 are examples of SCP polypeptides that, when expressed in a cell, inhibit the activity of wild-type SCP.

Anti-SCP Antibodies

[00133] In another aspect, the invention provides an anti-SCP antibody. The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin.

[00134] The antibody can be a polyclonal, monoclonal, recombinant, e.g., a chimeric or humanized, fully human,

non-human, e.g., murine, or single chain antibody. In a preferred embodiment it has effector function and can fix complement. The antibody can be coupled to a toxin or imaging agent.

[00135] A full-length SCP protein or, antigenic peptide fragment of SCP can be used as an immunogen or can be used to identify anti-SCP antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of SCP should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of SCP. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

[00136] In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding an SCP polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

[00137] A vector can include a SCP nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the

nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., SCP proteins, mutant forms of SCP proteins, fusion proteins, and the like).

[00138] The recombinant expression vectors of the invention can be designed for expression of SCP proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990).

Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

[00139] The invention further provides a recombinant expression vector comprising an SCP DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which

direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics, Vol. 1(1) 1986.

[00140] A host cell can be any prokaryotic or eukaryotic cell. For example, an SCP protein can be expressed in bacterial cells such as E. coli, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[00141] Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., SCP DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

Screening Assays

[00142] The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to SCP proteins, have a stimulatory or inhibitory effect on, for example, SCP expression or SCP activity, or have a stimulatory or inhibitory effect on, for example, the expression or

activity of a SCP substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., SCP genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

[00143] Compounds used in the methods described herein can be proteinaceous in nature, such as peptides (comprised of natural and non-natural amino acids) and peptide analogs (comprised of peptide and non-peptide components), or can be non-proteinaceous in nature, such as small organic molecules. The substance can also be a genetically engineered SCP protein with an altered amino acid sequence. These substances would be designed to bind to, or interact with the SCP protein based on the DNA or amino acid sequences of the SCP proteins described herein, or antibodies reactive with the SCP proteins described herein.

[00144] For example, a substance can be identified, or designed, that specifically interferes with the phosphatase activity of one, or more, SCP proteins thereby inhibiting RNA polymerase II holoenzyme activity. Monoclonal or polyclonal antibodies (e.g., the polyclonal antibodies described herein) specific for one, or more, of the SCP proteins can also be used to prevent, or inhibit, the SCP proteins from participating in the initiation of gene transcription.

[00145] In one embodiment, the invention provides assays for screening candidate or test compounds which are target molecules of a SCP protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a SCP

protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) Anticancer Drug Des. 12:145).

[00146] In one embodiment, an assay is a cell-based assay in which a cell which expresses a SCP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate SCP activity determined. Determining the ability of the test compound to modulate SCP activity can be accomplished by monitoring the bioactivity (i.e., phosphatase activity) of the SCP protein or biologically active portion thereof. The cell, for example, can be of mammalian origin.

Methods for Cell Differentiation

[00147] The invention encompasses methods for modulating or regulating the differentiation of a population of a specific progenitor cell into specific cell types comprising differentiating the progenitor cell under conditions suitable for differentiation and in the presence of one or more compounds of the invention. Alternatively, the stem or progenitor cell can be exposed to a compound of the invention and subsequently differentiated using

suitable conditions. As used herein, compound includes small molecules, RNA (e.g. siRNA), DNA, chemical compositions and antibodies.

[00148] The invention also encompasses the modulation of stem or progenitor cells in vivo, in a patient to be treated. Thus, one or more of the SCP1, SCP2 or SCP3 inhibitory compounds of the invention, alone or in combination, may be administered to a patient. In various embodiments, such compounds may be administered concurrently or serially in combination with, for example, stem or progenitor cells, the differentiation of which has been modulated using one or more of the compounds of the invention; with treated stem or progenitor cells and untreated stem or progenitor cells. The compound and any treated or untreated cells may be administered together or separately; in the latter case, the cells or the compound(s) may be administered first.

[00149] In a specific embodiment, the present invention provides methods that employ SCP1, SCP2 and/or SCP3 inhibitors to modulate and regulate nerve tissue regeneration.

[00150] In other embodiments, the methods of the invention may be used to regulate the differentiation of e.g., a neuronal precursor cell or neuroblast into a specific neuronal cell type such as a sensory neuron (e.g., a retinal cell, an olfactory cell, a mechanosensory neuron, a chemosensory neuron, etc.), a motoneuron, a cortical neuron, or an interneuron. In other embodiments, the methods of the invention may be used to regulate the differentiation of cell types including, but not limited to, cholinergic neurons, dopaminergic neurons, GABA-ergic neurons, glial cells (including oligodendrocytes, which

produce myelin), and ependymal cells (which line the brain's ventricular system). In yet other embodiments, the methods of the invention may be used to regulate the differentiation of cells that are constituent of organs, including, but not limited to, purkinje cells of the heart, biliary epithelium of the liver, beta-islet cells of the pancreas, renal cortical or medullary cells, and retinal photoreceptor cells of the eye.

[00151] As used herein, the term "stem cell" refers to a master cell that can reproduce indefinitely to form the specialized cells of tissues and organs. A stem cell is a developmentally pluripotent or multipotent cell. A stem cell can divide to produce two daughter stem cells, or one daughter stem cell and one progenitor ("transit") cell, which then proliferates into the tissue's mature, fully formed cells.

[00152] As used herein, "stem cell" includes embryonic and adult (somatic) stem cells. An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found.

[00153] Any mammalian stem cell can be used in accordance with the methods of the invention, including but not limited to, stem cells isolated from cord blood ("CB" cells), placenta and other sources. The stem cells may include pluripotent cells, i.e., cells that have complete differentiation versatility, that are self-renewing, and can remain dormant or quiescent within tissue. The stem

cells may also include multipotent cells or committed progenitor cells.

Examples

[00154] The alignment of three human proteins that are closely related to one another and have homology to the phosphatase domain of human FCP1 is shown in Figure 1A. All contain the signature motif $\Psi\Psi\text{D}\text{X}\text{D}\text{X}(\text{T}/\text{V})\Psi\Psi$. The full-length 261 aa protein is encoded by 7 exons; a shorter NH_2 terminal splice version of 214 aa is present in EST databases. SCP1 has ~20% homology to human FCP1 in the phosphatase domain while the 3 SCP proteins are >90% homologous in this region. SCP2/OS4 located on chromosome 12q13 was co-amplified with CDK4 in sarcomas (Su et al., (1997) Oncogene 15:1289-1294) and SCP3/HYA22 located on chromosome 3q22 was part of a large chromosome deletion in a lung carcinoma cell line. These represent a subset of proteins with putative CTD phosphatase-like catalytic domains found in plants, yeast, nematodes and arthropods. The Drosophila and Anopheles genomes each contain a single highly conserved SCP ortholog. The SCP proteins lack the BRCT domain present in FCP1 (Figure 1B).

[00155] The SCP1 protein was expressed as a GST-fusion and both SCP1 261 and SCP1 214 were assayed using PNOP as substrate. The pH optimum for SCP1 phosphatase activity is near 5 (Figure 2A). Phosphatase activity was Mg^{2+} -dependent and resistant to the phosphatase inhibitors okadaic acid and microcystin (Figure 2B). Ca^{2+} could not substitute for Mg^{2+} . Mutations of Asp95 to Glu (D95E) had little to no effect on phosphatase activity whereas mutating Asp97 to Asn (D97N) in conjunction with the D95E mutation completely abolished phosphatase activity (Figure 2B). SCP1 is thus a

class 2C phosphatase whose activity is dependent on acidic residues in the conserved DXD motif. SCP2 exhibited similar phosphatase activity (Figure 2B).

[00156] GST-CTDo and RNAP IIO were utilized as substrates and the activity of SCP1 was compared directly with that of FCP1. Recombinant CTDo (rCTDo) and RNAP IIO utilized as substrate in these experiments were prepared by the phosphorylation of purified GST-CTDa or RNAP IIA with casein kinase II (CKII) in the presence of [γ - 32 P]ATP followed by phosphorylation with MAPK2/ERK2 in the presence of excess unlabeled ATP. MAPK2/ERK2 was used in these initial experiments because it phosphorylates both GST-CTDa and RNAP IIA with comparable efficiency. FCP1 converts RNAP IIO to RNAP IIA in a processive manner (Figure 2C, lanes 7-12). Higher concentrations of FCP1 did not result in measurable dephosphorylation of rCTDo (Figure 2C, lanes 1-6). GST-SCP1.214 catalyzed the dephosphorylation of both RNAP IIO and GST-CTDo with comparable efficiency (Figure 2D). In contrast to FCP1, the SCP1 catalyzed dephosphorylation of RNAP IIO appears non-processive in that a number of phosphorylated intermediates are visible in SDS-PAGE. SCP1 is specific for dephosphorylation of the consensus repeat in that the phosphate at the CKII site is not removed. Mutant SCP1 (D95E, D97N) lacked activity on either substrate. SCP1 is a CTD phosphatase that acts on both RNAP IIO and rCTDo. SCP2 exhibits comparable CTD phosphatase activity when RNAP IIO is utilized as substrate (see e.g., Figure 4).

**SCP1 preferentially dephosphorylates Ser 5 of the CTD
heptad repeat**

[00157] To determine the specificity of SCP1 with respect to its ability to dephosphorylate specific positions within the consensus repeat, RNAP IIO isozymes were prepared *in vitro* by the phosphorylation of RNAP IIA with CTD kinases of known specificity. TFIIH, P-TEFb and MAPK2/ERK2 preferentially phosphorylate Ser 5 when synthetic peptides serve as substrate whereas Cdc2 kinase phosphorylates Ser 2 and Ser 5. Although the specificity appears relaxed when RNAP II serves as substrate, RNAP IIO prepared with Cdc2 kinase is clearly distinct from RNAP IIO generated by other CTD kinases. Results presented in Figure 3A indicate that RNAP IIO, prepared by the phosphorylation of RNAP IIA with distinct CTD kinases, exhibit a differential sensitivity to dephosphorylation with SCP1. SCP1 most efficiently dephosphorylates RNAP IIO generated by TFIIH and was unable to dephosphorylate RNAP IIO prepared with Cdc2 kinase. SCP1 was also unable to dephosphorylate RNAP IIO generated by Abl tyrosine kinase. The dephosphorylation of RNAP IIO isozymes prepared with P-TEFb, MAPK2/ERK2 and CTDK1/CTDK2 occurred at a reduced rate relative to that of RNAP IIO prepared with TFIIH. Furthermore, while the dephosphorylation reaction appears processive for RNAP IIO prepared by TFIIH, it is clearly non-processive for RNAP IIO generated by MAPK2/ERK2. In contrast FCP1 shows no preference for RNAP IIO generated by TFIIH and efficiently dephosphorylates RNAP IIO generated by Cdc2 kinase. These results indicate that SCP1 differs from FCP1 in substrate specificity, showing relative preference for the dephosphorylation of Ser 5 in the heptad repeat.

[00158] A synthetic 28 aa peptide containing 4 heptad repeats phosphorylated exclusively on Ser 2 or on Ser 5 was dephosphorylated in the presence of increasing amounts of SCP1. As shown in Figure 3B, SCP1 preferentially dephosphorylates the Ser 5 phosphopeptide compared to the Ser 2 phosphopeptide. This substrate specificity contrasts to that reported for FCP1 from *S. pombe* which preferentially dephosphorylates the Ser 2 phosphopeptide. Mammalian FCP1, within a comparable concentration range, did not act on either phosphopeptide. These results using synthetic phosphopeptide substrates confirm that SCP1 preferentially dephosphorylates Ser 5 phosphate of the CTD.

Effect of RAP74 on the activity of SCP1

[00159] The RAP74 subunit of TFIIF stimulates CTD phosphatase activity of FCP1. Furthermore, the domains of FCP1 that bind RAP74 are required for FCP1-dependent viability in *S. cerevisiae*. Therefore, it was of interest to determine if RAP74 can also influence the activity of SCP. CTD phosphatase activity was measured at low enzyme concentrations to more readily detect stimulatory effects of RAP74. As shown in Figure 4, RAP74 shifted the dose response curve for SCP1 catalyzed dephosphorylation of RNAP IIO to an approximately 10-fold lower concentration. The CTD phosphatase activity of the GST-fusion forms of SCP1 261, SCP1 214 and SCP2 were also enhanced by RAP74. In support of the conclusion that RAP74 stimulates the activity of SCPs, RAP74 bound directly to GST-SCP1 but not to GST. The binding and stimulatory effects of RAP74 suggest that TFIIF is important for optimal CTD phosphatase activity for both FCP1 and SCP1.

SCP1 nuclear localization

[00160] Although SCP1 lacks an obvious nuclear localization sequence, it is found in the nucleus. Immunofluorescence microscopy using a rabbit polyclonal anti-SCP1 antibody demonstrated nuclear localization of endogenous SCP1 in COS7 cells (Figure 5 B). Co-staining with DAPI for nuclear identification and with the early endosomal marker EEA1 for cellular detail confirmed the specific localization of SCP1 in nuclei (Figure 5 panels A and B).

[00161] Co-immunoprecipitation was used to assess the association of SCP1 with RNAP II. Sepharose-immobilized anti-SCP1 IgG 6703 was used to immunoprecipitate SCP1 from COS7 cells. Immunoprecipitates were resolved by SDS-PAGE and blotted with anti-RNAP II antibodies. As shown in Figure 5C, RNAP II was present in SCP1 immunoprecipitates indicating that SCP1 and RNAP II either interact directly or are in the same macromolecular complex. To determine whether SCP1 preferentially associated with either Ser 2 or Ser 5 phosphorylated RNAP II, lysates were prepared in the presence of EDTA, to inhibit phosphatase activity. SCP1 immunoprecipitates were then blotted with monoclonal antibodies specific for Ser 2 phosphate (H5) and Ser 5 phosphate (H14). Both forms of RNAP II were present in COS7 cell lysates. Ser 5 phosphate-enriched RNAP II appeared to be preferentially associated with SCP1 in immunoprecipitates as indicated by the ratios of co-immunoprecipitated RNAP II relative to the amount of RNAP II contained in the extract (Figure 5C).

SCP1 affects RNAP II transcription *in vivo*

[00162] To assess the effect of SCP1 on transcription *in vivo*, the activity of a variety of luciferase reporter gene

constructs was examined in the presence or absence of cotransfected SCP1. Targeting a Gal 4-DNA binding domain SCP1 fusion or the phosphatase-inactive Gal 4-SCP1 mutant upstream of a thymidine kinase promoter-luciferase reporter (Gal 4-TK-Luc) had no significant effect on transcriptional activity (Figure 6A). Untethered SCP1 in the presence of several reporter constructs had no significant effect on reporter gene expression whereas the inactive mutant resulted in a significant stimulation of expression from the ElALuc, pGL3-Luc and Gal4-TATA-Luc constructs (Figure 6B). The phosphatase-minus SCP1 mutant increased luciferase activity 1.5- to 6-fold.

[00163] In contrast, WT or phosphatase-inactive SCP1 affected reporter gene expression from a variety of regulated promoters. Luciferase activity from a Gal 4-TK-Luc reporter that was strongly stimulated by co-expressing a Gal 4-VP16 fusion protein (30-fold stimulation) was strongly inhibited by SCP1 (Figure 6C). In contrast, phosphatase-inactive SCP1 enhanced Gal 4-Vp16-stimulated activity about 2-fold.

[00164] Opposing effects of active SCP1 and the inactive mutant SCP1 were observed using a number of inducible promoter-reporter constructs. Ligand-activated T₃ receptor activity on a DR+4 TRE-TK-Luc reporter gene was inhibited by active SCP1 and enhanced by inactive SCP1 (Figure 6D). Similar results were obtained when the C-terminus of T₃R β was fused to the Gal 4-DNA binding domain (Gal 4-T₃R β C) and targeted to Gal 4-TK-Luc. SCP1 also inhibited dexamethasone-stimulated glucocorticoid receptor activity on a GRE-TK-Luc construct whereas mutant SCP1 significantly enhanced activity (Figure 6D). Finally, SCP1 inhibited ligand-activated PPAR γ receptor activity assayed on a PPAR γ

promoter response element and mutant SCP1 enhanced activity (Figure 6D). A similar response pattern was observed when the Gal 4-DNA binding domain was fused to the C-terminus of PPAR γ and targeted to Gal 4-TK-Luc. The same pattern of responses was observed in HEK293, COS-7 and CV-1 cells.

[00165] The competing effects of SCP1 and mutant SCP1 were further examined using a rat insulin promoter-luciferase construct. There is strong synergy on this promoter between the bHLH protein E47 and the LIM-homeodomain protein LMX1 which bind to adjacent DNA target sites (39,40). Co-expression of LMX1 and E47 enhanced luciferase activity 25-fold from the rat insulin 1 promoter (Figure 6E). When phosphatase-inactive SCP1 was held constant, increasing amounts of SCP1 inhibited luciferase expression (Figure 6E). In the presence of mutant SCP1, the SCP1 inhibition curve was right-shifted (~20% inhibition with 40 ng SCP1 plasmid plus 20 ng mutant SCP1 plasmid vs. >90% inhibition with 40 ng SCP1 plasmid alone (Figure 6E)). With a constant input of SCP1 plasmid, increasing amounts of mutant SCP1 not only blocked the inhibitory effects of SCP1, but enhanced activity significantly. The stimulatory effects of transfected phosphatase-inactive SCP1 are thus consistent with it acting as a dominant negative inhibitor of wild-type SCP activity.

[00166] Acquisition of the CTD of RNAP II allows extensive protein interactions and is thought to have been an important step in the evolution of complex patterns of regulated gene expression. Most importantly, the CTD can exist in multiple conformations thereby facilitating the recruitment of different multiprotein complexes at specific points in the transcription cycle. Both the site of phosphorylation within the consensus repeat, Ser 2 or Ser

5, and the extent of phosphorylation of the CTD control many aspects of RNAP II transcription, including the recruitment of RNAP II to the preinitiation complex, initiation, capping, elongation, splicing and polyadenylation. Ser 2 and Ser 5 within the consensus CTD repeat are essential residues and distinct CTD kinases catalyze phosphorylation at these sites (West and Corden, (1995) Genetics 140:1223-1233). To date, a single CTD phosphatase, FCP1, has been implicated in removing phosphates (for review see Lin et al., (2002) Prog. Nucl. Acid Res. Mol. Biol. 72:333-365).

[00167] SCP1 preferentially catalyzes dephosphorylation of RNAP IIO phosphorylated by TFIIH. RNAP IIO phosphorylated by P-TEFb and MAPK2/ERK2 are also dephosphorylated by SCP1 but at a reduced rate. RNAP IIO phosphorylated by Cdc2 kinase, which preferentially phosphorylates Ser 2 with some phosphorylation at Ser 5, is not a substrate for SCP1. The preferential dephosphorylation of Ser 5 by SCP1 was confirmed using a 4 heptad repeat peptide substrate. Interestingly, the specificity of SCP1 contrasts with that of *S. pombe* FCP1 which prefers Ser 2 with a similar peptide substrate (Hausmann and Shuman, (2002) J. Biol. Chem. 277:21213-21220). However, FCP1 dephosphorylates Ser 2 phosphates and Ser 5 phosphates with comparable efficiency when native RNAP IIO serves as substrate *in vitro* (Lin et al., (2002) J. Biol. Chem. 277:45949-45956). The relative specificity of FCP1 also differs from SCP1 in that unlike FCP1, SCP1 shows preference for the dephosphorylation of TFIIH phosphorylated RNAP IIO.

[00168] It is clear from the results presented in Figure 2C and Figure 2D that, when RNAP IIO phosphorylated with MAPK2/ERK2 is utilized as substrate, the specific activity

of SCP1 is substantially lower than that of FCP1. This difference in specific activity is in part due to the fact that MAPK2/ERK2 phosphorylated RNAP IIO is an especially poor substrate for SCP1 (Figure 3) whereas FCP1 dephosphorylates different isozymes of RNAP IIO with comparable efficiency. The amount of SCP1 required to dephosphorylate MAPK2/ERK2 phosphorylated RNAP IIO is 50 to 100 fold higher than that required to dephosphorylate TFIIF phosphorylated RNAP IIO. Furthermore, the BRCT domain in FCP1 that facilitates its interaction with RNAP II is absent from SCP1. The finding that SCP1 dephosphorylates rCTDo and RNAP IIO with comparable efficiency whereas FCP1 does not dephosphorylate rCTDo even at concentrations 100 times higher than that required to dephosphorylate RNAP IIO, indicate that the interaction of SCP1 and FCP1 with the CTD require different molecular interactions.

[00169] During the transcription cycle, protein complexes assemble and disassemble on the CTD in a dynamic and regulated manner. Ser 5 phosphorylation is detected primarily at the promoter region, whereas Ser 2 phosphorylation is seen in coding regions. Phosphorylation of Ser 5 facilitates recruitment of the capping enzymes and allosterically activates their activity (Pei et al., (2001) J. Biol. Chem. 276:28075-28082). Given the preference of SCP1 for phosphoserine 5, SCPs are candidates for acting early in the transcription cycle.

[00170] Like FCP1, SCP1 phosphatase activity is found in a complex with RNAP II and its activity is stimulated by RAP74. Mapping studies indicate that the C-terminal domain of FCP1 distal to the phosphatase domain interacts with TFIIF. A region near the C-terminus of SCP1 shares homology with the putative RAP74 interaction domain in CP1.

[00171] The results of reporter gene assays indicate that over-expression of either WT or mutant SCP1 can influence gene expression. The overexpression of mutant SCP1 activates transcription several fold from nearly all promoters examined (Figure 6B, C and D) whereas overexpression of WT SCP1 appears to selectively inhibit activated transcription from a variety of inducible promoter-reporter gene constructs (Figure 6D). Because mutant SCP1 is competitive with SCP1, the stimulatory effects are consistent with partial inhibition of endogenous SCP.

Extinction of SCP Expression in Differentiating Nervous Tissue

[00172] Northern analysis revealed that SCP1 is widely expressed with the highest levels observed in skeletal muscle and low to absent expression in brain (Fig. 7A). To determine whether a specific SCP family member was expressed in brain, probes specific for SCP 2 and SCP3 were also used. SCP2 and SCP3 expression was also very low in brain relative to other tissues indicating that expression of the entire SCP family is largely excluded from nervous tissue. These results confirm low expression of SCP1 and SCP2 in brain.

[00173] To examine the pattern of expression of SCPs in the developing nervous system, in situ hybridization was carried out in mice at e 10.5. SCP1 is widely expressed in cells surrounding the developing spinal cord and is expressed in proliferating neuroepithelium adjacent to the neural tube (Figure 7B). However, SP1 is absent from the differentiating spinal cord lateral to the proliferating zone where neuronal differentiation markers are expressed

(Figure 7C). Similarly SCP1 is expressed in proliferating neuro-epithelium adjacent to the 3rd ventricle but is absent from surrounding differentiated neuronal cells. This pattern of expression parallels that of REST/NRSF whose expression is widespread in non-neuronal tissue but excluded from neuronal tissues.

SCP1 is Found in a Complex with REST/NRSF at RE-1 DNA Elements

[00174] SCP1 co-immunoprecipitates with RNAP II suggesting it is part of a molecular complex with a subset of RNAP II molecules. The pattern of exclusion of SCPs from differentiated nervous tissue indicates that these phosphatases function with REST/NRSF to silence neuronal gene expression in non-neuronal tissues. The interactions between SCP1 and REST/NRSF were determined using co-immunoprecipitation. REST/NRSF immunoprecipitates contained SCP1; conversely SCP1 immunoprecipitates contained REST/NRSF (Figure 8A) indicating that the two proteins exist in a molecular complex in non-neuronal cells.

[00175] Chromatin immunoprecipitation (ChIP) with anti-SCP antibodies and PCR primers specific for the REST/NRSF binding elements of the Na⁺ channel II (SCN2A2), glutamate receptor (GRIN2A) and glutamic acid decarboxylase (GAD1) genes was used to determine that SCP1 was part of the REST/NRSF complex. As shown in Figure 8B, SCP is specifically associated with the REST binding sites of the SCN2A2, GRIN2A and GAD1 genes as confirmed by parallel immunoprecipitations using anti-REST/NRSF antibodies but not by control immunoglobulin. Anti-REST/NRSF and anti-HP1 antibodies similarly indicated these proteins did not

localize to the region of the 3' region of the GAD1 gene. Using antibodies to other components of the REST complex, ChIP analysis indicated localization of HDAC1, HP1 (hetero chromatin protein 1) and MeCp1 at the RE-1 element (Figure 8B). These proteins along with CO-REST are part of the REST/NRSF complex. These results indicate that SCP is a component of the REST/NRSF complex located at the RE-1 DNA binding elements of neuronal genes.

SCP1 Expression in Embryonic Stem Cells

[00176] P19 mouse embryonic stem cells can be induced to undergo neuronal differentiation by treatment with retinoic acid under defined cell culture conditions. Clonal P19 cell lines expressing SCP1, a mutant phosphatase-inactive D96E/D98N SCP1 that acts as a dominant negative, REST/NRSF or GFP as a control, were generated to determine the pattern of expression and effects of SCP1 on neuronal differentiation. Under defined conditions > 90% of viable P19 cells undergo morphological differentiation into neurons (Figure 9A, Figure 9C). Expression of REST/NRSF, which did not affect stem cell proliferation, prevented neuronal differentiation and most cells died under selective neuronal induction conditions (Figure 9F). Consistent with a requirement for REST/NRSF, whose expression is extinguished upon neuronal differentiation, SCP1 did not affect the extent of neuronal differentiation (Figure 9D). However dominant negative SCP1 increased the extent of precursor differentiation into neurons >2-fold (Figure 9E).

[00177] SCP1 and REST/NRSF are expressed in replicating P19 stem cells but the expression of both genes is extinguished upon differentiation into neurons.

Concomitantly cells acquire expression of the neuron-specific gene β -tubulin. These results indicate that both SCP and REST/NRSF expression are extinguished upon neuronal differentiation. Moreover blocking SCP effects in P19 stem cells by dominant negative SCP increased the fraction of stem cells that differentiated into neurons.

Suppression of SCP Enhances Neuronal Gene Expression

[00178] Although a Drosophila REST/NRSF neuronal gene silencing mechanism orthologous to that in seen mammals has not been defined, Drosophila also suppress neuronal gene expression in non-neuronal cells. Because examination of the fly databases revealed no P element insertions or specific deletions in the SCP locus, silencing RNA (siRNA dSCP) was used to "knock down" Drosophila SCP (dSCP) expression. The Drosophila genome contains a single SCP ortholog with high homology to human SCP1 (75% amino acid identity), making use of siRNA more feasible than in mammalian cells, which express 3 SCPs. Because the level of dSCP expression is relatively stable from the earliest times of analysis of Drosophila embryos there is likely a strong maternal component of dSCP mRNA during early development. The Drosophila gene product expressed as a GST-fusion protein in bacteria exhibited phosphatase activity similar to that described for human SCPs.

[00179] S2 cells, which were initially established from 20-22 hr., Drosophila embryos were treated with a 700 bp siRNA dSCP to decrease dSCP expression and effects on expression of a variety of neuronal and non-neuronal genes was achieved without obvious effects on S2 cell growth or morphology. dSCP mRNA was reproducibly decreased >80% by

24 hr. Decreasing dSCP had no effect on expression of glyceraldehyde phosphate dehydrogenase (GAPDH), ribosomal protein S35 or β -actin mRNAs. In contrast siRNA dSCP enhanced expression of a set of neuronal genes: the sodium channel II gene (NaChII), the glutamate receptor, ELAV, β -tubulin and glial cell missing (GCM) 2 to >10 fold. The mammalian orthologs of NaChII, glutamate receptor and β -tubulin are classical neuronal genes that contain RE-1 elements. Some gene transcripts could not be detected: synapsin, stathmin, choline acetyltransferase (ChoAcTR), neurofilament and a non-neuronal oxygenase. Myosin light chain kinase, which was robustly expressed in control S2 cells, was increased 2.5-fold when dSCP was suppressed. In S2 cells expression of a set of neuronal genes is thus markedly enhanced when dSCP is decreased suggesting dSCP acts to suppress their transcription. The effects of siRNA dSCP to increase expression of the glial specific gene ELAV indicates that dSCP acts to repress both neuronal and glial-specific gene expression. This is consistent with lack of expression of SCP in mammalian brain and spinal cord that contain both glial and neuronal elements.

[00180] Figure 1A shows the sequence alignment and relationship of 3 small phosphatases with FCP1. Bracket indicates the conserved signature motif and * indicate critical Asp residues involved in phosphatase activity. Previous descriptive names and chromosome locations are indicated. Multiple alignments were done using Clustal W algorithm with vector NTI Suite (Informax). Figure 1B provides diagrams of the domain structures of FCP1 and SCP proteins.

[00181] Figure 2A is an autoradiogram showing the pH optimum for SCP1 utilizing synthetic peptide substrates. GST-SCP1 214 (40 pmol) was incubated with 20 mM PN0P for 60 min at 30°C and phosphatase activity measured by the change in A_{410} . Figure 2B provides data showing the divalent metal ion requirement for SCP1 activity. The phosphatase activity of GST-SCP1 214 (40 pmol) was measured in the presence of 20 mM PN0P and varying concentrations of $[Mg^{2+}]$ or $[Ca^{2+}]$. Activity was also measured in the presence of 1-10 μ M okadaic acid and 1-10 μ M microcystin. The 10 μ M concentration is shown. Mutant SCP1 (D96E D98N) was inactive (- - -). SCP2 also exhibited phosphatase activity (\square).

[00182] Figures 2C and 2D show a CTD phosphatase assay of FCP1 and SCP1 on GST-CTDo and RNAP IIO prepared by MAPK2/ERK2. Increasing amounts of FCP1 or GST-SCP1 214 were assayed in the presence of 75 fmol GST-CTDo (lanes 1-6), 75 fmol RNAP IIO (lanes 7-12), or 75 fmol GST-CTDo and 75 fmol RNAP IIO (lanes 13-18). Both GST-CTDo and RNAP IIO substrates were prepared by the *in vitro* phosphorylation of CKII-labeled GST-CTDa or RNAP IIA by MAPK2/ERK2. All reactions were carried out in the presence of 7 pmol RAP74. CTD dephosphorylation of both GST-CTDo and RNAP IIO is shown by the increase in mobility of GST-CTDo to GST-CTDa and subunit IIO to IIA, respectively. The difference in the intensity of radiolabeled GST-CTDo and that of radiolabeled subunit IIO is not a reflection of a difference in the amount of substrates present, but of the higher efficiency with which CKII incorporates radiolabeled phosphates onto the most C-terminal serine of GST-CTDa compared to subunit IIA.

[00183] Figure 3A shows dephosphorylation of RNAP IIO prepared with various CTD kinases. Increasing amounts of GST-SCP1 214 were assayed in the presence of 3.7 fmol RNAP IIO prepared by CTDK1/CTDK2, TFIIH, P-TEFb, MAPK2/ERK2 and Cdc2 kinase. All reactions were carried out in the presence of 7 pmol RAP74. CTD dephosphorylation of RNAP IIO isoforms by GSTSCP1 214 is shown by an increase in mobility of subunit IIO to that of IIA. The results are summarized in the graph showing the percent of RNAP IIO remaining as a function of increasing SCP1 concentrations.

[00184] Figure 3B shows the effects of GST-SCP1 214 on a 28 aa peptide consisting of heptad repeats containing either Ser 5 phosphate or Ser 2 phosphate. The indicated amounts of SCP1 were incubated with the phosphopeptide substrate and phosphate released was measured as described infra.

[00185] Figure 4 shows the effect of RAP74 on CTD phosphatase activity of SCP1 and SCP2. Increasing amounts of the indicated forms of SCP1 and SCP2 were assayed in the presence of 14.4 fmol RNAP IIO prepared by TFIIH. Reactions were carried out in the presence and in the absence of 7 pmol RAP74.

[00186] Figures 5A and 5B show cells co-stained for the endosomal marker EEA1 using mouse anti-EEA1 and Alexa Fluor 594 conjugated goat anti-mouse (red). Nuclei were detected with DAPI (blue) (5A). Immunofluorescence microscopy detection of endogenous SCP1 using rabbit polyclonal IgG 6307 and Alexa Fluor 488 conjugated goat anti-rabbit IgG (green) (5B). Second antibodies alone served as a control in all cases.

[00187] Figure 5C shows co-immunoprecipitation of RNAP II and endogenous SCP1. Extracts from untransfected COS7 cells were immunoprecipitated using sepharose-immobilized anti-

SCP1 IgG 6703 or sepharose-immobilized control IgG.

Immunoprecipitates were resolved on SDS-PAGE and blotted using anti-RNAP II antibody (8WG16) and with the Ser 2 phosphate epitope specific antibody H5 and the Ser 5 phosphate epitope specific antibody H14. RNAP II present in COS7 lysates is shown on the left (5% load) and the relative ratio of each form of RNAP II in SCP1 immunoprecipitates to that in lysates is given.

[00188] Figure 6A shows the effect of targeted SCP1 261 on reporter gene expression. Gal4-DNA binding domain-SCP1 fusion protein expression plasmids were cotransfected in HEK293 cells with a Gal 4-TKLuc reporter plasmid and luciferase gene expression quantitated. In all panels, results of triplicate transfections are shown +/- SD. Data is expressed as fold-activation experimental/control). Figure 6B shows the effect of SCP1 261 and mutant SCP1 261 on basal promoter activity. The indicated reporter plasmids were cotransfected with SCP1 or phosphatase-inactive SCP1. Figure 6C shows differential effects of SCP1 261 and phosphatase-inactive SCP1 261 on Gal 4-VP16 stimulated gene expression. The indicated amounts of SCP1 or mutant SCP1 expression plasmids were cotransfected in HEK293 cells along with Gal 4-VP16 expression and Gal 4-TK-Luc reporter plasmids and luciferase activity was quantitated. Figure 6D shows the effect of SCP1 261 and phosphatase-inactive SCP1 261 on ligand activated receptor activity. The indicated receptor expression plasmids were cotransfected with their cognate promoter-reporter plasmids with or without SCP1 or mutant SCP1 expression plasmids. Cells were treated or untreated with receptor-specific ligands as described infra. Figure 6E shows the competitive effects of mutant SCP1 261 with SCP1 261. The

indicated concentrations of SCP1 and mutant SCP1 expression plasmids were cotransfected with LMX1 and E47 expression plasmids and the rat insulin 1 promoter-luciferase reporter gene. Luciferase activity was measured as described.

[00189] Figure 7A shows Northern blot analysis of the expression of SCP1 in human tissues. Figure 7B shows in situ hybridization analysis of expression of SCP1 in e 10.5 mouse cervical spinal cord. SCP1 is widely expressed in cells surrounding the developing spinal cord and in proliferating neuroepithelium adjacent to the neural tube (open arrow). SCP1 expression is absent from the differentiating spinal cord lateral to the proliferation zone (closed arrow). Figure 7 C provides in situ analysis of the expression of isl-1. Isl-1 is expressed in ventral motor neurons (closed arrow) and dorsal sensory neurons (open arrow), areas of the developing spinal cord where SCP1 is not expressed.

[00190] Figure 8A provides data indicating that SCP1 and REST/NRSF co-immunoprecipitate. HEK 293 cell extracts were immunoprecipitated using anti-SCP (upper panel) or anti-REST/NRSF (lower panel) antibodies, immunoprecipitates were resolved on SDS PAGE and associated proteins were identified by Western blotting using anti- REST/NRSF and anti- SCP1 antibodies. Figure 8B shows chromatin immunoprecipitation using anti-SCP antibody. ChIP assays of HEK 293 cells using 6703 anti-SCP, anti-REST/NRSF, anti HDAC1, anti-HP1 or control IgG. PCR primers (see Table 1) specific for the RE-1 elements of GAD1, GRINA 2A, SCN2A2 genes and for the 3' intron-exon region of GAD1 were used for RT-PCR. Upper panel: lanes 1-6 = RE1 element of the GAD 1 gene; lanes 7-11 = 3' region of GAD1 gene; lanes 1 and 7 = load (1%); lanes 2 and 8; control IgG; lanes 3 and

9, anti-SCP; lanes 4 and 10, anti-REST/NRSF; lanes 5 and 11 anti-HDAC1; lane 6, anti-HP1. Lower panel: lanes 1-6 = RE1 element of GRINA2 gene; lanes 7-11 RE1 element of SCN2A gene; lanes 1 and 7 = load (1%); lanes 2 and 8, control IgG; lanes 3 and 9, anti-SCP; lanes 4 and 10, anti-REST/NRSF; lane 6, anti-HP1; lane 11, anti-HDAC1.

[00191] Figure 9A through 9F shows SCP and REST/NRSF effects on neuronal differentiation of P19 cells. WT P19 cells and clonal lines expressing GFP (vector control), SCP1, mutant phosphatase-inactive SCP1 or REST/NRSF were induced to differentiate into neuron like cells (NLC) by treatment with retinoic acid and growth in selective medium a, undifferentiated; B differentiated P19 cells. P19 cells; C, differentiated GFP expressing P19 cells; D, differentiated SCP1-expressing P19 cells; F, differentiated REST/NRSF-expressing P19 cells. Numbers of NLC per field are shown. *p=.001 compared to WT 19 cells; **p=.008 compared to WT P19 cell using student's t-test.

[00192] In addition, the inventors have found that silencing of SCP enhances expression of neuronal and glial genes. Figure 10 shows the quantitation of transcripts using real time quantitative RT-PCR. S2 cells were either untreated (-) or treated (+) with siRNA dSCP for 24 hr. Total RNA was prepared, DNase treated and primer pairs specific for the coding sequence of each gene were used for qPCR using SYBR green chemistry.

Materials

[00193] SCP1 and SCP2 were obtained as EST clones from Resgen. The full-length cDNA for SCP1 (261 aa, accession BE300370), the cDNA encoding the spliced variant of SCP1 (214 aa, accession L520011) and SCP2 (accession AL520463)

were subcloned into EcoRI-XhoI sites of pGEX4T-1 and pCDNA3Flag vectors by PCR. The D96E, D98N mutant of SCP1 261 (nucleic acid SEQ ID NO:9 encoding amino acid SEQ ID NO:10) and the corresponding mutant of SCP1 214, D48E and D50N (nucleic acid SEQ ID NO:11 encoding amino acid SEQ ID NO:12), were generated by QuikChange (Stratagene). The amino acid sequence of SEQ ID NO:10 is identical to that of SEQ ID NO:2 except that the aspartic acid (D) at position 96 in SEQ ID NO:2 has been changed to glutamic acid (E) in SEQ ID NO:10 and the aspartic acid (D) at position 98 of SEQ ID NO:2 has been changed to asparagine (N) in SEQ ID NO:10. In addition, the amino acid sequence of SEQ ID NO:12 is identical to that of SEQ ID NO:8 except that the aspartic acid (D) at position 48 in SEQ ID NO:8 has been changed to glutamic acid (E) in SEQ ID NO:12 and the aspartic acid (D) at position 50 of SEQ ID NO:8 has been changed to asparagine (N) in SEQ ID NO:12. GST fusions were purified by glutathione-sepharose chromatography and SCP1 261 was generated by cleavage at the thrombin site encoded in the vector. Recombinant FCP1 was expressed and purified as described previously (23).

[00194] Human recombinant casein kinase II (CKII) and mouse recombinant MAPK2/ERK2 were obtained from Upstate Biotechnology. Human CTDK1/CTDK2 were purified as described by Payne and Dahmus (28). Human TFIIH was obtained as described (29). Human P-TEFb was partially purified from HeLa S-100 extract by chromatography on Heparin-Sepharose (Amersham Biosciences), DEAE 15HR (Millipore) HiTrap S and Phenyl-Superose (both from Amersham Biosciences). P-TEFb was dialyzed against 25 mM Hepes, pH 7.9, 20% glycerol, 25 mM KCl, 0.1 mM EDTA, 1 mM DTT, 1 mM PMSF. Human recombinant Cdc2 kinase was

purchased from New England Biolabs. Rabbit anti-SCP1 IgG was prepared by ammonium sulfate fractionation and protein G-sepharose chromatography. RNAP II antibodies (8WG16, H5 and H14) were obtained from Covance.

[00195] **Preparation and Purification of ^{32}P -RNAP IIO Isozymes and ^{32}P -GST-CTDo:** Calf thymus RNAP IIA was purified by the method of Hodo and Blatti (30) with modifications as described by Kang and Dahmus (31). Specific isozymes of ^{32}P -labeled RNAP IIO were prepared by phosphorylation at the most C-terminal serine (CKII site) in the largest subunit of purified RNAP IIA with recombinant CKII and [K - ^{32}P]ATP, followed by CTD phosphorylation in the presence of 2 mM ATP with either purified CTDK1/CTDK2, TFIIF, P-TEFb, recombinant MAPK2/ERK2 or recombinant Cdc2 kinase. The RNAP IIO isozymes were individually purified over a DE53 column with a step elution of 500 mM KCl (28). Because only the most C-terminal serine is labeled with ^{32}P and lies outside the consensus repeat, dephosphorylation by CTD phosphatase results in an electrophoretic mobility shift in SDS-PAGE of subunit IIO to the position of subunit IIA without the loss of label. Similarly, ^{32}P -labeled GST-CTDo was prepared from GST-CTDa by CKII followed by MAPK2/ERK2. GST-CTDo was purified over a glutathione-agarose column with a step elution of 15 mM glutathione.

[00196] **Phosphatase Assays:** PN0P reaction mixtures (200 μl) containing 50 mM Tris-acetate, pH 5.5, 10 mM MgCl_2 , 0.5 mM DTT, 10% glycerol, 20 mM PN0P and recombinant proteins were incubated for at 30°C for 1 hr. The reactions were quenched by adding 800 μl of 0.25 N NaOH. Release of pN0 was determined by measuring A_{410} .

[00197] N-terminal biotinylated CTD phosphopeptides, comprised of 4 tandem repeats YSPTSPS and containing

phosphoserine at position 2 or position 5; were synthesized (Alpha Diagnostics, San Antonio, TX). Phosphatase reaction mixtures (50 μ l) containing 50 mM Tris-acetate, pH 5.5, 10 mM MgCl₂, 0.5 mM DTT, 10% glycerol, 25 μ M of phosphopeptide and wild type or mutant SCP1 were incubated for 60 mins at 37°C. The reactions were quenched by adding 0.5 ml of malachite green (Biomol). Phosphate release was measured at A₆₂₀ and quantified relative to a phosphate standard curve.

[00198] CTD phosphatase assays utilizing RNAP IIO and GST-CTDo as substrate were performed as described previously (32) with minor modifications. Reactions were performed in 20 μ l of CTD phosphatase buffer (50 mM Tris-HCl, pH 7.9, 10 mM MgCl₂, 20% glycerol, 0.025% Tween 80, 0.1mM EDTA, 5 mM DTT) in the presence of 20 mM KCl. Each reaction contained specified amounts of GST-CTDo and/or RNAP IIO and was carried out in the presence of 7 pmol RAP74. Reactions were initiated by the addition of FCP1 or SCP1 and incubated at 30°C for 30 minutes. Assays were terminated by the addition of 5X Laemmli buffer, and RNAP II subunits and GST-CTD were resolved on a 5% SDS-PAGE gel. The gel images were developed by autoradiography and scanned by Molecular Dynamics Image Scanner Storm 860 in the phosphor screen mode. Data were quantitatively analyzed by ImageQuant software.

[00199] **Tissue Culture and Transfections:** Human 293, COS7 and CV1 cells were grown at 37°C in DMEM supplemented with 10% normal calf serum (BRL). Sub-confluent cells were transfected in 6 well tissue culture dishes using Effectene (Qiagen) according to the manufacturer's instructions. Reporter and activator plasmids (100 ng each) and Flag SCP1 (80 ng) or its mutant were used per well. For T3 and PPAR γ

transfections, 20 ng RXR plasmid was also added. The amount of ligands used are as follows: 100 nM T3, 1 μ M PPARK609843 (Ligand Pharmaceuticals), 100 nM dexamethasone. LMX1 and E47 expression plasmids (100 ng) were cotransfected with 100 ng of the rat insulin promoter-luciferase reporter construct with the indicated concentrations of SCP1 and phosphatase-minus SCP1 expression plasmids. The total amounts of transfected DNA was kept constant by the addition of empty vector. Cells were harvested 48 hrs after transfections and cellular extracts were assayed for luciferase activity using Luciferase Assay System (Promega) according to the manufacturer's instructions.

[00200] **Immunofluorescence:** Cells grown on coverslip were fixed in 2% paraformaldehyde, neutralized and blocked using 2.5% FCS/PBS. Rabbit polyclonal IgG 6703 was used at 1:100 dilution, followed by goat anti-rabbit IgG H+L chains conjugated to Alexa Fluor 488 (1:250). Mouse anti EEA1 was used at 1:1000 followed by goat anti-mouse IgG conjugated to Alexa Fluor 594 (1:250). Omission of primary antibodies was used as negative control. The coverslips were viewed using the Zeiss Axiophot which is equipped with a Hamamatsu Orca ER firewire camera that runs on Improvision Openlab 3.0.9 software.

[00201] **Immunoprecipitations:** For immunoprecipitation experiments, 75% confluent COS7 cells from a 10 cm dish were harvested in lysis buffer (PBS containing 1% NP40, 1 mM DTT and protease inhibitors). Lysates were incubated with 20 μ l sepharose-conjugated anti-SCP1 (6703) IgG at 4°C for 6 hr. Beads were washed with PBS and the complexes were evaluated by western blotting using specific anti-RNAP II antibodies. Rabbit anti-SNX1 antibody was used as control IgG.

[00202] Northern and in-situ: Multiple tissue blot (Clontech no.636818) was used to determine the distribution of human SCP isoforms in adult tissues. Specific SCP1 cDNA was prepared by PCR, labeled using AlkPhos Labeling kit (Amersham) and used as probe. The same blot was stripped and rehybridized with specific SCP2 and SCP3 cDNA probes. The prehybridization, hybridization, washing and deprobing were done according to manufacturer's instructions (Clontech no. 636831). β -actin was used as internal control.

[00203] In-situ Co-immunoprecipitation and ChIP: For Co-IP experiments, 293 cells were harvested in lysis buffer (PBS containing 1%NP40, 1mM DTT and protease inhibitors) and incubated with either anti-SCP or anti-REST/NRSF antibodies overnight at 4°C. 15 μ l of Protein A/G-Sepharose was then added to the lysates and incubated at 4°C for 3h. Beads were washed with PBS and the complexes were analyzed by Western blotting using either anti-REST/NRSF or anti-SCP antibodies.

[00204] ChIP was performed according to a modification of the method of Spencer et. al. (Methods 31:67-75,2003). About 0.7×10^6 cells were used for each ChIP experiment. Cells were cross-linked with 1% formaldehyde for 30min, washed twice with cold PBS, resuspended in lysis buffer (1%SDS, 10mM EDTA, 50mM Tris-HCl pH 8.0, 1Xprotease inhibitor cocktail (Roche)) and sonicated for 15s pulses at 40% with a Braun-Sonic sonicator. The lysates are clarified by centrifugation at 10000 rpm for 10 min at 40°C in a microcentrifuge. One-tenth of the total lysate was used as input control of genomic DNA. Supernatants were collected and diluted in buffer (1%TritonX-100, 2mM EDTA, 150mM NaCl, 20mM Tris-HCl pH 8.0, protease inhibitor cocktail) followed

by immunoclearing with 1 µg salmon sperm DNA, 10 µl rabbit IgG and 20 µl protein A/G-sepharose (Santa Cruz Biotechnology) for 1 h at 4°C. Immunoprecipitation was performed overnight at 4°C with 2 µg of each specific antibody. Precipitates were washed sequentially for 10 min each in TSE1 buffer (0.1%SDS, 1%Triton-X100, 2m M EDTA, 150 mM NaCl, 20 mM Tris-HCl pH 8.0), TSE2 (TSE 1 with 500 mM NaCl) and TSE3 (0.25M LiCl, 1% NP40, 1% deoxycholate, 1mM EDTA, 10 mM Tris-HCl pH 8.0). Precipitates were then washed twice with TE buffer and extracted with 1% SDS containing 0.1M NaHCO₃. Eluates were pooled and heated at 65°C for 6h to reverse the formaldehyde cross-linking. DNA fragments were purified with Qiagen Qiaquick spin kit. For PCR, 1 µl of a 25 µl DNA extraction was used.

[00205] Anti-Rest Ab (P18 and C15), anti-HP1 (D15), anti-HDAC1 (H11) anti-MeCP2 (H300) are from Santa Cruz Biotechnology Inc. Anti-SCP1 was prepared by immunizing rabbits using GST-SCP1 as antigen.

[00206] Inhibition of SCP expression by siRNA: S2 cells were propagated in 1x Schneider's Drosophila media/10% FBS, at room temperature. For dsRNA production, individual DNA fragments approximately 700 bp in length, spanning nt 121-853 of dSCP1 to be "knocked out" were amplified by using PCR. Each primer used in the PCR contained a 5' T7 RNA polymerase binding site (GAATTAATACGACTCACTATAGGGAGA). The PCR products were purified by using MicroSpin S-400 columns (Pharmacia). The purified PCR products were used as templates for transcription using a MEGASCRIP^T kit (Ambion). The dsRNAs were annealed by incubation at 65°C for 30 min followed by slow cooling to room temperature. The dsRNA products were ethanol-precipitated and resuspended in

water. Concentration of dsRNA was determined using a spectrophotometer at OD₂₆₀. To induce RNA interference, S2 cells were diluted to a final concentration of 1×10^6 cells/ml in Drosophila expression system (DES) serum-free medium (Invitrogen). One milliliter of cells was plated per well of a six-well cell culture dish (Corning). 12 µg dsRNA was added directly to the cells. This was followed immediately by vigorous agitation. The cells were incubated for 30 min at room temperature followed by addition of 2 ml of 1× Schneider's media containing FBS. The cells were incubated for an additional 3 days to allow for turnover of the target protein.

[00207] **Quantitative RT-PCR:** Total RNA from S2 cells was prepared using RNeasy kit (Qiagen). RNA was DNase 1 treated after which the RNA concentration was determined at OD₂₆₀ nm with a spectrophotometer. Primer concentrations for the RT-PCR were optimized to yield the lowest threshold cycle (Ct) and maximum Rn while minimizing non-specific amplification. One µg of total RNA was used for each RT-PCR using the ABI Prism 7700 Sequence Detection System and SYBr Green chemistry according to manufacturer's instructions (number 4310179). To quantitate the target sequence amounts, serial dilutions (0-10 ng) of pcDNA3-SCP1 was used as template to generate a standard curve. The Ct values of standard template was plotted against the log of the corresponding copy number. The standard curve was then used to determine the amounts of the target sequences in the unknown samples, achieved by determining the corresponding Ct values. The entire process of determining the Ct values and constructing the standard curve(s) was performed as part of the data analysis using the SDS v.1.7

software. For each sample, the amplification plot and the corresponding dissociation curves were examined.

[00208] P19 Differentiation: The P19 cultured cell-lines used include a) P19 stably expressing flag-hSCP1; b) P19 stably expressing flag-hSCP1(D96E, D98N) phosphatase-inactive mutant; P19 stably expressing EGFP; and d) P19 stably expressing REST/NRSF.

[00209] These cell lines were maintained in α -MEM/10%FBS with 400 mg/ml G418. To induce neuronal differentiation, P19 cells were allowed to aggregate in bacterial grade petri-dishes (Fisher) at a seeding density of 1×10^5 cells/ml in the presence of 1×10^{-6} M all-trans retinoic acid. After 3 days of aggregation, cells were dissociated into single cells by 0.05% trypsin-0.53 mM EDTA (Gibco). Trypsin was removed by centrifugation and the cells were plated in 6 cm tissue culture dishes (Nunc) at a density of 1×10^5 cells/cm² in Neurobasal medium /N2 supplement (Invitrogen) in the presence or absence of 1 μ g/ml fibronectin (Invitrogen). The cells were cultured for 5 days after which average cell numbers/field were determined. Cells were either lysed to prepare total RNA (Rneasy kit, Qiagen) or stained with TuJ1 antibody (Babco).

[00210] For immunostaining, cells were fixed for 10mins with 4% paraformaldehyde. TuJ1 antibody was used at 1:2000 and secondary antibody used was FITC-conjugated anti-mouse (1:3000).

[00211] RT-PCR was done using heat-stable rTth DNA Polymerase according to manufacturer's instructions (Novagen).

[00212] Embryonic stem cells, as their name suggests, are derived from embryos. Specifically, embryonic stem cells

are derived from embryos that develop from eggs that have been fertilized in vitro - in an in vitro fertilization clinic - and then donated for research purposes with informed consent of the donors. They are not derived from eggs fertilized in a woman's body. The embryos from which human embryonic stem cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst. The blastocyst includes three structures: the trophoblast, which is the layer of cells that surrounds the blastocyst; the blastocoel, which is the hollow cavity inside the blastocyst; and the inner cell mass, which is a group of approximately 30 cells at one end of the blastocoel.

[00213] All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

Sequences

SCP1 nucleotide sequence (SEQ ID NO:1)

```

1 atggacagct cggccgtcat tactcagatc agcaaggagg aggctcgggg cccgctgcgg
61 ggcaaagggtg accagaagtc agcagcttcc cagaagcccc gaagccggggg catcctccac
121 tcactcttct gctgtgtctg ccgggatgat ggggaggccc tgctgtctca cagcggggcg
181 cccctgcttg tggaggagaa tggcgccatc cctaagacct cagtccaata cctgtccct
241 gaggccaagg cccaggactc agacaagatc tgcgtggtca tcgacctgga cgagacctg
301 gtgcacagct cttcaagcc agtgaacaac gcggaattca tcatccctgt ggagattgat
361 ggggtggtcc accaggtcta cgtgttgaag cgtcctcatg tggatgagtt cctgcagcga
421 atgggcgagc tctttgaatg tgtgctgttc actgctagcc tcgccaagta cgcagacca
481 gtagctgacc tgctggacaa atggggggcc ttccggggcc ggctgtttcg agagtccctgc
541 gtcttccacc gggggaacta cgtgaaggac ctgagccggg tgggtcgaga cctgcggcgg
601 gtgctcatcc tggacaattc acctgcctcc tatgtcttcc atccagacaa tgcgtgaccg
661 gtggcctcgt ggtttgacaa catgagtgc acagagctcc acgacctcct ccccttcttc
721 gagcaactca gccgtgtgga cgacgtgtac tcagtgtca ggcagccacg gccagggagc
781 tag

```

SCP1 amino acid sequence (SEQ ID NO:2)

```

MDSSAVITQI SKEEARGPLR GKGDQKSAAS QKPRSRGILH SLFCCVCRDD GEALPAHSGA
PLLVEENGAI PKTPVQYLLP EAKAQDSDKI CVVIDLDEL VHSFVKPVNN ADFIIPVEID
GVVHQVYVLK RPHVDEFLQ MGELFECVLF TASLAKYADP VADLLDKWGA FRARLFRESC
VFHRGNYVKD LSRLGRDLRR VLILDNSPAS YVFHPDNAVP VASWFDNMSD TELHDLPLFF
EQLSRVDDVY SVLRQPRPGS

```

SCP2 nucleotide sequence (nucleotides 306-1157 = SEQ ID NO:3)

```

1 gccatttcct cctcttgttt tcactccgga ttctccatgt tggacccaaa ctgaggagcc
61 cggagctgcc gctgggggat cggggcggg ggcaccggg ggagccgctg cccgggccc
121 ccgccctttg tacaggccgc ctcccttccc ggtccgggga ggaaacgaga ggggggatgt
181 gaacagctgt ggaagtcgga gtctcgggag ccggagcggg ccccgccca ggccccccag
241 cccagcccag ccgcgcgcgc cgcctgtcct cccgtccagc cagcccgggc ccgcgggatt
301 gttagatgga acacggctcc atcatcacc aggcgcggag ggaagacgcc ctggtgtctca
361 ccaagcaagg cctggtctcc aagtcctctc ctaagaagcc tcgtggagct aacatcttca
421 aggccctttt ctgctgtttt cgcgcccagc atgttgcca gtcaagttcc tccactgagc
481 tcgctgcgta taaggaggaa gcaaacacca ttgctaagtc ggatctgtct cagtgtctcc
541 agtaccagtt ctaccagatc ccagggacct gcctgtctcc agaggtgaca gaggaagatc
601 aaggaaggat ctgtgtggtc attgacctcg atgaaacct tgtgcatagc tcctttaagc
661 caatcaacaa tgctgacttc atagtgccta tagagattga ggggaccact caccaggtgt
721 atgtgtctaa gaggccttat gtggatgagt tcctgagacg catgggggaa ctctttgaat
781 gtgttctctt cactgccagc ctggccaagt atgccgacct tgtgacagac ctgctggacc
841 ggtgtggggt gttccgggccc cgcctattcc gtgagtcttg cgtgttccac cagggtgtct
901 acgtcaagga cctcagccgc ctggggaggg acctgagaaa gacctcctc ctggacaact
961 cgctgtcttc ttacatattc caccgagaga atgcagtgcc tgtgcagtc tggtttgatg
1021 acatggcaga cactgagttg ctgaacctga tcccaatctt tgaggagctg agcggagcag
1081 aggacgtcta caccagcctt ggggcagctg cgggccctt agcctgccct gcttccaagc
1141 gacggccatc ccagtagggg actttccac actgtgcctt tacgatcagc gtgacagagt
1201 agaagctgga gtgcctcacc acacggcccc gaaacagcgg gaagtaactg gaaagagctt
1261 taggacagct tagatgccga gtgggcgaat gccagaccaa tgatacccag agctacctgc
1321 cgccaacttg ttgagatgtg tgtttgactg tgagagagtg tgtgtttgtg tgtgtgtttt
1381 gccatgaact gtggccccag tgtatagtgt ttgagtgagg gagaagctga aagaccaaga
1441 ctcttcccaa gttagcttgt ctctctcct gtcaccctaa gagccactga gttgtgtagg

```

```

1501 gatgaaract attgaagact ccattgccaa accatggcct ttcctcagtg ttgtaaggcc
1561 tatgccaagg ataaaggaag ggtatgcctt tgggtactcc aggcatacac ctttctgaaa
1621 tccttctcca gccagctgct gcagacaaaa gatcacattt ctgggaagat gagaacttgt
1681 ttccagacca gcatccagtg gccatcaggt cttgtggccc aaaggctatg cttgcctccg
1741 gctgagtgcc tgggataggc cttttctatg tctcccaag gctgggtgct tgagcctgcc
1801 ttcctcacca cctagccata gtctcaaacc tgtggggaag gaggttttct ccctgcccgg
1861 gaagaggaca gataactgat ttccgttctt ttgactgtgt tttaaaattc tctttctaaa
1921 cacagagtgt tgggcctggt ttgtttctga caaagttaca gtccctgggc tgtaatgaat
1981 gtccggcgcg ctgggggttg agggaaaaga caaatcctca aagcgtggac gtgtgtcccc
2041 atggcttggt gatcagctaa gctcgggatc atttccataa gtctgctttt cagggattct
2101 ctctgggtgc tggtgcaagg acttctgttc caaaggctgg gaaaaactaa gctgtcccag
2161 cccctcccat ttcttgggca gggctctttt cctgttgtgt cttccccag ggcctgtcct
2221 gtaaccgagct ctgtctgttc cagcctacat ccttctctgg tgttgctttt cctcttaagg
2281 gcctcagaac tcttgctctt cctggggtga gggggaatga gtgttcttga catgtgacag
2341 cctaattgct atgttttctg cctctggtta caggagttag tgagccccct agacctgcac
2401 tctgggtgct tctgtctaac aaaggttctt aatagtgaat gctttaaaat taaagtcatc
2461 acgaaatgga agttttccca ggggtgaaaa taagaggaag tgctgctgta attgggagca
2521 caaggggctt cccaaaaagg agccccacct cagcatcact gccttaatcg tggcctccct
2581 ggggtgggtg gggttctctc ctccctccct cctcctcctt ggggtgggag ggcgtcctg
2641 ttcccatctc tgtgttccct ggaggcaggt atcacaaagc atttgtgaat tgctttaggt
2701 gcagggacac caccactca ggactcttcc ccatcatccc ttccattgct acaccctaga
2761 tccagcctca ggaactaaca agttktgaga aaagcaggtg gttagagcag agcttctgtg
2821 tctcagcggt ggctggctgg catttttctc tagcgttgtg gtgccacctt ccttcttgt
2881 cccaaggtta taaggccttg tctttctctt tggaatcata aagtggaaac gagtccccag
2941 aactcatgtg ghcatttccg acagcatcac tccccgggtc ctatgggggt ccggtgtacc
3001 taaagggaga aggaccccat gtgctagcca gaaatatact gtctcttgaa ggaaagcagg
3061 agctcagact cttagagcca gctgtggctt cggacccaag gcctgacctt ggtgctatc
3121 ctaatatattg aggaggggccc tcttctccaa gccccacctt aagggttagc cttggacaa
3181 atcttgtgccc gtctaggccc agccaggctt ttctgactaa ataagcaata agaggctcta
3241 agctgactga gttgcaagga ccctttccgc cctcccttgg atctccatgt ttctccagat
3301 ggcggaagag catgtgccac cccctttcct aacagacttg tccaagtgtt tggcgtggga
3361 cccatgacca aagcccagga tggcttggtg ggagtgtccc tgctgcatct gcatgaagcc
3421 cctgcttttt aggcctcact cccatcagaa ccctgectgc ccacctgcaa ctccccccca
3481 acaatgccat tcccacttgc ccagagaaag ctactcggcc aaacctagcc aggggttgtt
3541 cttgtggacc agagccagcc tagtcattat ttgctgtcgg gtttccagtt tcaccgtgtg
3601 ttaggggtgag ggatgattgt aaaatttgct cctcaaagga atcaggccag actcaatttt
3661 gggagggcaa gacagggagg aggcgcttc atccagact ctcttctagg gcttcccacc
3721 atcagccccct cccacttgag actggtcttt gggaggcaat aggccaccat gcctggtcag
3781 caccaattca agccatgcc ggaatctgcc tacctgccag gttcagttct ttaagggtgc
3841 ctcttcaggg acacagtgtg tctctctgat tgggcttcta aatcaaaagc ctgatgttcg
3901 tgtccctctc atagggggag ctttggacac aggaccagtt tggaaaaggg tcaggttaagg
3961 gtttccactc tgcacattgt agaggaaca ctctgtaggc ccatgggtcc cttactagag
4021 aggttgagtg aatttgcctt cagttaacat gggaccttct gtttagcttc ctcttgcttc
4081 ccaaagattt taagcatttt gtaaatgtat aaactcacct ctggtaacag tggcccagac
4141 gctgctttgt gctaaaagca tgggaaatgt aaaggcagtc tttctctggg aaatggatgc
4201 tattctattc tgctgcccc accgttctct gaggcctcat ttagaaagaa aatccccctc
4261 gaaggctgtc tggcaccag tgtcctagcc aggccaaagta tatgagaaag gtaagtccat
4321 ttcccccttc aggtcctcag tggattactt aacctctgct gtccctcggt ccttttttcc
4381 taaacgggtt tagttctgtc ttttttctcc ttttttctaa atgctggtaa atatttacat
4441 tcagccaggg aagaggagg cagaggtcgg gccagctgcc ccattctttt aacgttgtag
4501 ggcctgccc tggagcggac cctcctcttt gggcctctg agcttttttg cttatcatgt
4561 tccatttctg gccgctttcc ccttcaaga tggcatttgg agggtagggg atctgcttcc
4621 cactgtgact ggcctatggg attctgacta ccttgcttac agattcatgg tttgataaat
4681 ttgttgtatt ccaaaacttg aaatgcagga cgccattaag tgtctgttta ttttttgga
4741 atatttgtat tacttacaat taattaataa aagtgggttt aaaaaacctt tccagggaaa
4801 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa

```

SCP2 amino acid sequence (SEQ ID NO:4)

MEHGSIIITQARREDALVLTQGLVSKSSPKKPRGRNIFKALFCC
FRAQHVQSSSSTELAAYKEEANTIAKSDLLQCLQYQFYQIPGT
CLLPEVTEEDQGRICVVIDLDET LVHSSFKPINNADFIVPIEIE
GTHQVYVLKRPYVDEFLLRMGELFECVLFTASLAKYADPVTDL
LDRCGVFRARLFRESCVFHQGCYVKDLSRLGRDLRKTLLDNLSP
ASYIFHPENAVPVQSWFDDMADTELLNLIPFEELSGAEDVYTS
LGAAAGPLACPASKRRPSQ"

SCP3 nucleotide sequence (nucleotides 1-798 = SEQ ID NO:5)

```

1 atggacggcc cggccatcat caccacaggtg accaaccacca aggaggacga gggccgggtg
61 ccggggcgcg ggcagaaagc ctcccagtcg aacgtcagct taaagaagca gaggagccgc
121 agcatcctta gctccttctt ctgctgcttc cgtgattaca atgtggaggc ccccccaccc
181 agcagcccca gtgtgcttcc gccactgggtg gaggagaatg gtgggcttca gaagccacca
241 gctaagtacc ttcttcacaga ggtgacgggtg cttgactatg gaaagaaatg tgtggctcatt
301 gatttagatg aaacattggg gcacagttcg tttaagccta ttagtaatgc tgattttatt
361 gttccgggtg aaatcgatgg aactatacat caggtgtatg tgctgaagcg gccacatgtg
421 gacgagttcc tccagaggat ggggcagctt tttgaatgtg tgctctttac tgccagcttg
481 gccaaagtat cagaccctgt ggctgacctc ctgacccgct ggggtgtgtt ccgggcccgg
541 ctcttcagag aatcatgtgt ttttcatcgt ggaactacg tgaaggacct gagtcgcctt
601 gggcggggagc tgagcaaagt gatcattgtt gacaattccc ctgcctcata catcttccat
661 cctgagaatg cagtgcctgt gcagtcctgg ttcgatgaca tgacggacac ggagctgctg
721 gacctcatcc ccttctttga gggcctgagc cgggaggacg acgtgtacag catgctgcac
781 agactctgca ataggtagcc ctggcctctg cctgcctccc gcctgtgcac tctggaacct
841 ctggcctcag gggacctgc

```

SCP3 amino acid sequence (SEQ ID NO:6)

MDGPAIITQVTNPKEDGRLPGAGEKASQCNVSLKKQRSRSILS
SFFCCFRDYNVEAPPPSPSVLPPLVEENGGLQKPPAKYLLPEV
TVLDYGKKCVVIDLDET LVHSSFKPISNADFIVPVEIDGTIHQV
YVLKRPYVDEFLLRMGQLFECVLFTASLAKYADPVADLLDRWGV
FRARLFRESCVFHRGNYVKDLSRLGRELSKVIIVDNPSASYIFH
PENAVPVQSWFDDMTDTTELLDLIPFEGLSREDDVYSMLHRLCNR

SCP1 214 nucleotide sequence (nucleotides 1-642 = SEQ ID NO:7)

```

1 atgatgggga ggcctgcct gctcacagcg gggcgcccct gcttgtggag gagaatggcg
61 ccatccctaa ggcagacccc agtccaatac ctgctccctg aggccaaggc ccaggactca
121 gacaagatct gcgtgggtcat cgacctggac gagaccctgg tgcacagctc cttcaagcca
181 gtgaacaacg cggacttcat catccctgtg gagattgatg ggggtgtcca ccaggctctac
241 gtgttgaagc gtcctcacgt ggatgagttc ctgcagcgaa tgggcgagct ctttgaatgt
301 gtgctgttca ctgctagcct cgccaagtac gcagacccag tagctgacct gctggacaaa
361 tggggggcct tccggggccc gctgtttcga gactcctgcg tcttccaccg ggggaactac
421 gtgaaggacc tgagccggtt ggggtcgagac ctgcggcggg tgctcatcct ggacaattca
481 cctgcctcct atgtcttcca tccagacaat gctgtaccgg tggcctcgtg gtttgacaac
541 atgagtgcac cagagctcca cgacctcctc ccttcttcg agcaactcag ccgtgtggac
601 gacgtgtact cagtgtcag gcagccacgg ccaggagct agtgagggtg atggggccag
661 gacctgcccc tgaccaatga taccacacac tcctcccagg aagactgccc aggcctttgt

```

721 taggaaaacc catggggccgc cgccacactc agtg

SCP1 214 amino acid sequence (SEQ ID NO:8)

mmgrpcllta grpclwrrma pslrqtpvqy llpeakagds dkiavvidld etlvhssfkp
vnnadfiipv eidgvvhqvy vlkrphvdef lqrmgelfec vlftaslaky adpvadlldk
wgafrarlfr escvfhrqny vkdlrlgrd lrrvlildns pasyvfhpdn avpvaswfdn
msdtelhdll pffeqlsrvd dvysvlrqr pgs

SCP1 D96E, D98N mutant nucleotide acid sequence (SEQ ID NO:9)

1 atggacagct cggccgtcat tactcagatc agcaaggagg aggctcgggg cccgctgcgg
61 ggcaaagggtg accagaagtc agcagcttcc cagaagcccc gaagccgggg catcctccac
121 tcaactcttct gctgtgtctg ccgggatgat ggggaggccc tgctgtctca cagcggggcg
181 cccctgcttg tggaggagaa tggcgccatc cctaagacc cagtccaata cctgctccct
241 gaggccaagg cccaggactc agacaagatc tgcgtggtca tcgaactgaa cgagaccctg
301 gtgcacagct ccttcaagcc agtgaacaac gcggacttca tcatccctgt ggagattgat
361 ggggtggtcc accagggtcta cgtgttgaag cgtcctcatg tggatgagtt cctgcagcga
421 atgggcgagc tctttgaatg tgtgctgttc actgctagcc tcgccaagta cgcagaccca
481 gtagctgacc tgctggacaa atggggggcc ttccggggcc ggctgtttcg agagtcctgc
541 gtcttccacc gggggaacta cgtgaaggac ctgagccggg tgggtcgaga cctgcggcgg
601 gtgctcatcc tggacaattc acctgcctcc tatgtcttcc atccagacaa tgctgtaccg
661 gtggcctcgt ggtttgacaa catgagtac acagagctcc acgacctcct ccccttcttc
721 gagcaactca gccgtgtgga cgacgtgtac tcagtgtca ggcagccacg gccagggagc
781 tag

SCP1 D95E, D9N mutant amino acid sequence (SEQ ID NO:10)

MDSSAVITQI SKEEARGPLR GKGDQKSAAS QKPRSRGILH SLFCCVCRDD GEALPAHSGA
PLLVEENGAI PKTPVQYLLP EAKAQSDSKI CVVIELNETL VHSSFKPVNN ADFIIPVEID
GVVHQVYVLK RPHVDEFLQR MGELFECVLF TASLAKYADP VADLLDKWGA FRARLFRESC
VFHRGNYVKD LSRLGRDLRR VLILDNSPAS YVFHPDNAV P VASWFDNMSD TELHDLPPFF
EQLSRVDDVY SVLRQPRPGS

SCP1 214 D48E, D50N mutant nucleotide acid sequence (SEQ ID NO:11)

1 atgatgggga ggcctgcct gctcacagcg gggcgccct gcttgtggag gagaatggcg
61 ccateccctaa ggcagacccc agtccaatac ctgctccctg aggccaaggc ccaggactca
121 gacaagatct gcgtggtcat cgaactgaac gagaccctgg tgcacagctc cttcaagcca
181 gtgaacaacg cggacttcat catccctgtg gagattgatg ggggtggtca ccaggctctac
241 gtgttgaagc gtcctcacgt ggatgagttc ctgcagcgaa tgggcgagct ctttgaatgt
301 gtgctgttca ctgctagcct cgccaagtac gcagacccag tagctgacct gctggacaaa
361 tggggggcct tccggggccc gctgtttcga gagtccctgcg tcttccaccg ggggaactac
421 gtgaaggacc tgagccggtt gggtcgagac ctgcggcggg tgctcatcct ggacaattca
481 cctgcctcct atgtcttcca tccagacaaat gctgtaccgg tggcctcgtg gtttgacaac
541 atgagtgaca cagagctcca cgacctctc ccttcttctg agcaactcag ccgtgtggac
601 gacgtgtact cagtgtcag gcagccacgg ccaggagct ag

SCP1 214 D48E, D50N mutant amino acid sequence (SEQ ID NO:12)

MMGRPCLLTA GRPCLWRRMA PSLRQTPVQY LLPEAKAQDS DKICVVIELN ETLVHSSFKP
VNNADFIIPV EIDGVVHQVY VLKRPHVDEF LQRMGELFEC VLFTASLAKY ADPVADLLDK
WGAFRARLFR ESCVFHRGNY VKDLSRLGRD LRRVLILDNS PASYVFHPDN AVPVASWFDN
MSDTELHDLI PFPEQLSRVD DVYSVLRQPR PGS

SCP1 nucleic acid sequence on chromosome 2 (SEQ ID NO:13)

ctggagcgcg gcaggaaccc ggcccggccc gcctcccagt ccgcctagcc gcgcccgtcc
cagaagtggc gaaagccgca gccgagtcga ggtcacgccg aagccgttgc ccttttaagg
gggagccttg aaacggcgcc tgggttccat gtttgcaccc gcctcgccgg aaggaaactc
catgttgtaa caaagtttcc tccgcgcccc ctccctcccc ctcccccta gaacctggct
cccctcccct ccggagctcg cggggatccc tccctccac ccctcccctc cccccgcgc
cccgattccg gccccagccg ggggggaggg cgggcgcccc ggccagagtc cggccggagc
ggagcgcgcc cggccccatg gacagctcgg ccgtcattac tcagatcagc aaggaggagg
ctcggggccc gctgcggggc aaaggtaccg gggctgcggg gagggggccg aagccggggc
gccgtgggag gagagaagg gcccggatct tccccagggg agccgcgcgc gccgcccggg
gcggccgcct tagctgtgcc cgaagctccc agcccagagag ggagcagggg gagagtttga
actcagagga ggctcagaga cgcggggcgg ggcctggcgc ctttggggcg ctccgtgtccg
ctcgaggtga ggaaactgag gcaggaatag agagggaact ccttcggggg tttcctggca
ggcattgcgt ggtgcattgg cgcgccccc ccatggcgcc caatgggggt gtgagatggg
ggagctgagg agggcgccca tgggcccacc gctgagactc cgccccacc cccaccccca
cccccccggg ctgcggtccg gtaggggtctt gggagggggc gccgaggtga cagcaggctg
gggaggcttg gagggatctc ccgccaacac acagctacgt tccccacaaa cttcgcgtca
cgctgaggag cgcgcgcccc ctccgaggga cagagaggac ggccgggact tccaagagtc
gcttggcgcc cgcggggaga gtcgtgcgcc tagtggggac gcaccacccc gcaaagcctc
gccgccccga cgaggtcgcc tccccagcg tggctggggc ggggtggggg ggtctgtctt
ctccttttcc ccgtgtggac ctccaggtctt ggcgctgccc cccaggtctg cccaccctcg
cctgggtctg gctgccccgg aactgagggc aaggtggaaa ggctagttgc agggggccgg
aggggggtgg ggtgggagg gttatctgtca atcaggctgc tgggctccag gtcggaggtc
tgggccccgg agggcaaaaca gatggccact ggacactggc cccaggccgc gggactgcac
ccctgcctct gggcccagcc gcagttagga cttcgtaccc acgggggtgg agaggatgga
gggagggcag ggggtgactg cctgggtccc caggccctgg ctgtcctgag caggggtgct
caggtacagg ggggtcagga ggcaccgcaa tggggctgat cagcagcagt catggaggct
gtgagaggca gggagagagc accccaggac ctcccttctc aggccacgca ctccctatgt
gggcgcctta atacctgcta gacctatttg tctgggagct gcaggagcct tggagttgat
tgtggagccc tgacaggggc gtttcagaga aagtccaggag ctgccttcgt gtgtctggat
gaagggggcca cggcaagatc ctccctggccc aggggttcac acctgggcac acatgcagga
ttctgcaggc cagtgtgcac cgagcctcca acttgtcct ccctacttca ggtgaccaga
agtcagcagc tccccagaag ccccgaagcc ggggcatcct ccaactcactc ttctgctgtg
tctgccggga tgatggggag gccctgcctg ctcacagcgg ggcgcccctg cttgtggagg
agaatggcg catccctaag gtgcgtgggg gccagggtgg gccacggggg cacctggact
cagtccttcag ggcttttagg gaaggggctc ctgactgagc ttttcaggat ggacttgcag
acctgaaagt gcagagtagg aggggtggcag cctcccctgc caggccctgc ccactgtggg
gaaactgaat tctccctcat aagtggaaagc ttttttctac cttgggtttt agagaggtct
caaagagcca agaggcctac ccaagcccta gagctggcag gggcaaaagt gggaaagggg
aagtatctgt tcctggggcc tggggttcct ctggagacgg ctagggggag aagcctgcgt
gggaggaagg accaggcccc gagagaggca ccccagccag ccccgccctc cctacagcag
accccagtc aatacctgct ccctgaggcc aaggcccagg actcagacaa gatctgcgtg
gtcatcgacc tggacgagac cctgggtgac agctccttca aggtggggcc tgcctcaacag
ccctcagccc ggggtctcggg gggcatcccc caccctggcc tgggagggag gtgtgtgctg
gacccccatgc cctggggctc ctccccaac tccagcagct cttttccccc cagagccagt
gaacaacgcg gacttcatca tccctgtgga gattgatggg gtggtccacc aggtgagggc

caggaagagg	cagtgggtggg	cttggcatct	gcctccagac	cctaggctct	ttccaccaat
ccggagcgcc	tcggatggga	attggataca	tgtggaatgt	cagaggccca	gagagggtgt
gagacttgtc	ccaaagtcac	acagaacctc	aagggtctgt	gctgactcca	agcctgcaga
gtgggctcct	cctctaggct	ccccgtgct	gtgctccctc	gccccaccct	gcccgggacc
cagttcaagt	aattcaggat	aggttgtgtg	ctgtccagcc	tgttctccat	tacttggctc
ggggaccggg	gccctgcagc	cttgggggtga	gggggctgcc	cctggattcc	tgcactaggc
tgaggttgag	gcaggggaa	ggattgggaa	ttagggacct	cgtgaggtag	gactggccag
tggagtggaa	gttttgatcg	ttttctggcg	gggggtgggt	acagtttccc	cagcagtggg
cagggtagct	ggccaagcgg	agcctgcggg	cccagttctc	ttcctgtgcg	cctctgcctc
cctggcccat	gccctgccag	ccctcggcca	ccccacact	gccccactgg	cccgcagccc
cctcactggc	ccgcccccca	ggtctacgtg	ttgaagcgtc	ctcatgtgga	tgagttcctg
cagcgaatgg	gcgagctctt	tgaatgtgtg	ctgttactg	ctagcctcgc	caaggtgagc
cccacagggg	tcccggggga	accctgccct	cctacctacc	tcccgcctgc	agcccagtga
acctgcggcg	cccaggatga	cccacctcct	gctcccagta	cgcagaccca	gtagctgacc
tgctggacaa	atggggggcc	ttccggggcc	ggctgtttcg	agagtccctgc	gtcttccacc
gggggaacta	cgtgaaggac	ctgagccggg	tgggtcgaga	cctgcggcgg	gtgctcatcc
tggacaattc	acctgcctcc	tatgtcttcc	atccagacaa	tgctgtgagt	gcgggctgga
ctgggactgg	gacaggagct	gagacccagg	aagggtcag	tccattcagg	ccaccttggc
ctcttgagtc	cccagttggg	gggtgggtgc	cctcccagtc	cttctgtcat	tcatttgcctg
tgctgcggcg	ccactcccc	catccacctg	ccctgtagcc	atatggtctt	ttcccctcgc
acaaagcaga	gcatctgcca	tgcacagggg	ccccacagg	gcaacggagt	ttggaaagt
tcaatttttc	gaattgccag	ttgtgacct	ctgatggccc	acagaattaa	tttagtgggt
tctgattggg	aattttaaca	aaatgaaata	gaatagaaaa	tatccggtcg	ggtgcagtgg
ctcatgcctg	taatcccagc	actttgggaa	gctgaggtgg	gcaggtagct	gagcccagta
gttcaagacc	agcctcggca	acatagtga	accttatgtc	tacaaaaaat	acaaaaacta
gccaggcggt	gtggcgcatg	cctggagtcc	cggctatgca	gaaggctgag	gtaggagtat
cgcttgagcc	ctggaggcag	aggctgtggt	gagccaagat	tgtgccactg	cactctagcc
tgggcaacag	agcaagaccc	tgcctcaaaa	aaaaaaaaaa	gtatccaagt	gcttcgcaca
gataagggtta	ggaatttgtga	agcttttgca	ttgttacgtt	ataaatgtgt	tttccctggg
attgctgtca	aaaaagtttg	aacactgtgg	gtgaggggtt	ttcagaaact	gcatgatctg
agtagtggct	acatagggct	ggcctggaaa	ttctgcaccc	aggaccacct	gccccctcga
tcttcttaca	cccacttccc	caggtaccgg	tggcctcgtg	gtttgacaac	atgagtgaca
cagagctcca	cgacctcctc	cccttcttcg	agcaactcag	ccgtgtggac	gacgtgtact
cagtgtctcag	gcagccacgg	ccaggagct	agtgaggggtg	atggggccag	gacctgcccc
tgaccaatga	taccacaccc	tcctcccagg	aagactgccc	aggcctttgt	taggaaaacc
catgggcccgc	cgccacactc	agtgccatgg	ggaagcgggc	gtctccccc	ccagccccac
caggcggtgt	aggggcagca	ggctgcactg	aggaccgtga	gctccaggcc	ccgtgtcagt
gccttcaaac	ccctccccct	attctcaggg	gacctggggg	gcccctgctg	ctgctcccc
tttctgtctc	tgtccatgct	gccatgttcc	tctgtgtcca	aattgggccc	cttggcccc
tccggttctg	cttccctggg	gcagggttcc	tgccttgagc	cccagttctg	ggaacgggtg
acatcaagtg	ccttgcatag	agccccctct	tccccgcccc	gctttccccc	gggcacagct
ctaggctggg	aggggagaac	cagccccctc	ccctgcccc	cctcctccct	tgggactgag
agggccccct	ccaacctttg	cctctgcctt	ggagggaggg	gaggtctgtt	accactgggg
aaggcagcag	gagtctgtcc	ttcaggcccc	acagtgcagc	ttctccaggg	ccgacagctg
agggtgtctc	cctgcacatc	ccaagcaatg	acctcagaat	tctgccttaa	ccagccccgg
ggcttggtctc	ccccagctct	gagcgtgggg	gcataggcag	gacccccctt	gtggtgccat
ataaatatgt	acatgtgtat	atagattttt	aggggaagga	gagaggggaag	ggtcagggtta
gagacacccc	tccttgccc	ctttcctggg	cccagaagt	ggggggaggg	agggaaagga
tttttcaaat	ttttaaac	ctattttctg	aatggaacaa	gctgggccaa	ggggcccagg
ccctgtcctc	tgtccctcac	acccttttgc	tccgttcatt	cattcaaaaa	aacatttctt
gagcaccttc	tgtgcccagc	atatgctagg	cccaccagct	aagtgtgtgt	ggggggtctc
tacgccagct	catcagtgcc	tccttgcccc	tccttcaccc	gtgccttttg	gggatctgta
ggaggtggga	ccttctgtgg	ggtttggggg	tctccaggaa	gcccagacaa	gctgtcccc
tccccgtgct	caaccatct	cctacagccc	cctgcctgat	cccctgctgg	ctgggggagc
ctccaggat	atcctgcctt	ccaactgttt	ctgaagcccc	tcctcctaac	atggcgattc
cggagggtcaa	ggccttgggc	tctccccagg	gtctaaccgt	taaggggacc	cacataccag
tgccaagggg	gatgtcaagt	ggtgatgtcg	ttgtgtctcc	ctccccaga	gcgggtgggc

```

gggggggtgaa tatggttggc ctgcatcagg tggccttccc atttaagtgc cttctctgtg
actgagagcc ctagtgtgat gagaactaaa gagaaagcca gaccctatc ctgcttctgt
ggttattgcg ggggacttca gcaagtggg tgtgtgcctt gcacctgcg ctgccgtggg
ccccccccc gcttcagcac acctagagg ctggttggtg agggagggg tgcccggccc
tcgacacttc aggtgggaag ggcagcgtca gagcacaat ttgagcctcc aggctgtgct
cgtctacgtc ttcccgcctc gggtatgtgg tctgcaaat ggagatgtgc cctattggca
ggactaatta agtgcctgga cacagacgac aggatactag tagctggaaa gcaaaattcg
aaggcctggg taggggcagt cctggaatgc ggcgggggag ggggcgtggc ctctgcctg
gagcagagg ggggggctt tgcggctccg aaggcagagg cggggagcgg ggcgaggctc
tggttgagg ctccagcggc agaacttgtt ggcctgggtg cggcgggctc cggcgctgg
ctctgccggg cggcctgggt ggggccggcg ccgggggctc gcccccccg cccctctgcg
gcctctgagc agccattggc cgcgcccccg ccccaattcc cgccccgccc cgcgtccggg
aggcacttcc ttgcgaaac cgcgcggccc caggcgccg caggaaatgc cctcccgccg
tccccagcca gcctttgctt gcttcccacg ccagcgcta gaggcctccc tgtcctcgcg
gacgcaggaa ctccccggg gctggaaaga tggggccac ctcactcacc cctttcccg

```

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

What is claimed is

1. An isolated nucleic acid molecule selected from the group consisting of:

- a) a nucleic acid molecule consisting of a nucleotide sequence which is at least 80% identical to the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11;
- b) a nucleic acid molecule comprising a nucleotide sequence which is at least 80% identical to the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11;
- c) a nucleic acid molecule which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12;
- d) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12;
- e) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12 with 0 to 50 conservative amino acid substitutions; and
- f) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12 wherein the nucleic acid molecule hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 1, 3, 5, 7, 9 or 11, or a complement thereof, under stringent conditions.

2. An isolated nucleic acid molecule selected from the group consisting of:

- a) the cDNA deposited with ATCC as Accession Number BE300370;
- b) the cDNA deposited with ATCC as Accession Number AL520011; and

c) the cDNA deposited with ATCC as Accession Number AL520463,

or a complement thereof.

3. A nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11.

4. A nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11.

5. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence is at least 90% identical to SEQ ID NO:1, 3, 5, 7, 9 or 11.

6. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence is at least 95% identical to SEQ ID NO:1, 3, 5, 7, 9 or 11.

7. A vector containing the nucleic acid of claim 1, 2, 3 or 4.

8. A host cell containing the vector of claim 7.

9. The host cell of claim 8, wherein the host cell is a bacterial, yeast, insect or mammalian cell.

10. A method of producing a polypeptide, the method comprising culturing the host cell of claim 8 in a culture, expressing the polypeptide encoded by the nucleic acid in the cultured host cell, and isolating the polypeptide from the culture.

11. An isolated polypeptide selected from the group consisting of:

a) a polypeptide consisting of an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12;

b) a polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12;

c) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12 with 0 to 50 conservative amino acid substitutions;

d) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 80% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11; and

e) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4, 6 or 8, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 1, 3, 5, 7, 9 or 11, or a complement thereof, under stringent conditions.

12. An isolated polypeptide selected from the group consisting of:

a) the polypeptide encoded by the cDNA insert deposited with ATCC as Accession Number BE300370;

b) the polypeptide encoded by the cDNA insert deposited with ATCC as Accession Number AL520011; and

c) the polypeptide encoded by the cDNA insert deposited with ATCC as Accession Number AL520463.

13. A polypeptide comprising the amino acid sequence of

SEQ ID NO:2, 4, 6, 8, 10 or 12.

14. A polypeptide consisting of the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10 or 12.

15. The isolated polypeptide of claims 11, 12, 13 or 14, wherein the polypeptide is a phosphatase or a phosphatase inactive mutant.

16. The isolated polypeptide of claim 15, wherein the phosphatase is a serine phosphatase.

17. The isolated polypeptide of claim 16, wherein the serine phosphatase is a small C-terminal domain phosphatase (SCP) that dephosphorylates RNA polymerase II.

18. The isolated polypeptide of claim 15, wherein the serine phosphatase dephosphorylates serine 5 within the C-terminal binding domain (CTD) of RNA polymerase II.

19. The polypeptide of claim 18, wherein the phosphatase is small CTD phosphatase-1 (SCP1), small CTD phosphatase-2 (SCP2), or small CTD phosphatase-3 (SCP3).

20. The isolated polypeptide of claim 11, wherein the amino acid sequence comprises 0 to 30 conservative amino acid substitutions.

21. The isolated polypeptide of claim 11, wherein the amino acid sequence comprises 0 to 10 conservative amino acid substitutions.

22. The isolated polypeptide of claim 11, wherein the amino acid sequence is at least 90% identical to SEQ ID NO:2, 4, 6, 8, 10 or 12.
23. The isolated polypeptide of claim 11, wherein the amino acid sequence is at least 95% identical to SEQ ID NO:2, 4, 6, 8, 10 or 12.
24. An antibody that selectively binds to a polypeptide of claim 11, 12, 13 or 14.
25. The antibody of claim 24, wherein the antibody is polyclonal or monoclonal.
26. A method of promoting differentiation of a non-neuronal cell in to a cell of the nervous system, the method comprising:
- a) contacting the cell with a nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:12; and
 - b) expressing the polypeptide in the cell.
27. The method of claim 26, wherein the non-neuronal cell is a stem cell.
28. The method of claim 26, wherein the stem cell is an embryonic stem cell.
29. The method of claim 26, wherein the cell of the nervous system is a neuron, a sensory neuron, a motoneuron, an interneuron, a glial cell, a microglial cell or an

astrocyte.

30. The method of claim 26, wherein the nucleic acid molecule is an expression vector.

31. The method of claim 30, wherein the nucleic acid molecule is a viral genome.

32. A method of inhibiting differentiation of a non-neuronal cell in to a cell of the nervous system, the method comprising:

- a) contacting the cell with a nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8; and

- b) expressing the polypeptide in the cell.

33. A method of promoting RNA polymerase II associated transcription in a cell, the method comprising:

- a) contacting the cell with a nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:12; and

- b) expressing the polypeptide in the cell.

34. A composition comprising an inhibitor of small CTD phosphatase (SCP) gene expression, wherein the inhibitor is selected from the group consisting of:

- a) a small molecule inhibitor of gene expression;
- b) an anti-sense oligonucleotide; and
- c) a small interfering RNA molecule (siRNA or RNAi).

35. The composition of claim 34, wherein the inhibitor of SCP gene expression specifically binds to a polynucleotide selected from the group consisting of:

- a) a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, 5 and 7;
- b) a complement of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, 5 and 7;
- c) a reverse sequence of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, 5 and 7;
- d) a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, 4, 6 and 8;
- e) a complement of a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, 4, 6 and 8; and
- f) a reverse sequence of a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:2, 4, 6 and 8.

36. The composition of claim 34, wherein the cell is a stem cell.

36. A method of promoting the differentiation of a non-neuronal cell in to a cell of the nervous system, the method comprising contacting the non-neuronal cell with the composition of claim 34 in a sufficient concentration to inhibit the expression of a small CTD phosphatase (SCP).

37. A method of promoting the differentiation of a non-neuronal cell in to a cell of the nervous system, the

method comprising contacting the non-neuronal cell with the antibody of claim 24 in a sufficient concentration to inhibit the activity of a small CTD phosphatase (SCP).

38. A method for identifying a compound which modulates the activity of a polypeptide of claim 11, the method comprising:

a) contacting a polypeptide of claim 11 with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

39. A method of modulating the differentiation of a mammalian stem cell comprising contacting the stem cell with a compound that modulates SCP1, SCP2 or SCP3 activity, under conditions suitable for differentiation of said stem cell.

40. The method of claim 1, wherein the compound inhibits SCP1, SCP2 or SCP3 activity.

41. A method of transplanting a mammalian stem cell or progenitor cell to a patient in need thereof, the method comprising: (a) contacting the stem cell or progenitor cell with a compound that inhibits SCP1, SCP2 or SCP3 activity to produce a treated stem cell or progenitor cell; and (b) transplanting the treated stem cell into said patient.

42. An in vitro method to modulate the differentiation state of a stem cell, the method comprising: (i) contacting the stem cell with at least one inhibitory RNA molecule

(RNAi) comprising a sequence of a gene, or the effective part thereof, selected from the group consisting of SCP1, SCP2 and SCP3; (ii) providing conditions conducive to the growth and differentiation of the cell treated in (i); and optionally (iii) maintaining and/or storing the cell in a differentiated state..

ABSTRACT

Nucleic acids, polypeptides and methods are provided for regulating the phosphorylation state of the C-terminal domain (CTD) of RNA polymerase II (RNAP II). Also provided are methods for regulating cell differentiation.

10374699.doc

Table 1

Fly primers for qRT-PCR

Name of gene	5' sequence	3' sequence	Accession no.
SCP1	5' atgggcgaactatacagatgcgttc 3'	5' ctgtctgctgctggttcaacatgg 3'	CG5830
GAPDH	5' atcaacgacaacttcgagatcgtcg 3'	5' gcggttgagtagccaaactcgtg 3'	CG12055
ribosomal protein S35	5' atgtcgtcttgcaaaaactaagc 3'	5' ttataggatatcttcgatttcggc 3'	CG5497
beta-actin	5' tgaagatcctcaccgagcgcggcta 3'	5' gaccggactcgtcactactcctgctg 3'	NM_079486
Na Channel II	5' cagctggctgcgggagtagcggtcc 3'	5' tgcgcagctcgcccatgtagacctg 3'	CG9071
synapsin	5' gagctgtcgttgagctttggcg 3'	5' cgctgggattggggaagaaggtc 3'	CG3985
cholineAcetylTransferase	5' actggcctattactactggtc 3'	5' ccgtaaaaccgcgcgattaaagt 3'	CG32848
ELAV	5' caacgaagccgagcgcgcatccag 3'	5' tggcatggtcacgaatccgaatc 3'	CG4396
beta-tubulin	5' gcaacaactgggccaagggtcattac 3'	5' ctggcatgaacatctgctgggtcag 3'	CG9277
Neurofilament H	5' gcctccaagagcacgactacaaag 3'	5' cgatcagaagtgatcgcggtccta 3'	CG7421
peptidyl-glycine oxygenase	5' ctgcgaat caagtacctt gtgctgc 3'	5' ccctggctgaagcagaactcatg 3'	CG3832
myosin-light-chain-kinase	5' ctgcctcgcacctcagaacgac 3'	5' tatggcataaaagggtgtggccattc 3'	CG1915
GCM	5' caacggaactaacggcgcctccgag 3'	5' gttctcgccatcgttgagatctgc 3'	CG12245
nMDAR	5' ctgcgaattgtctcctgttg 3'	5' cgtacatgaggtagaccctgga 3'	CG14793

Mouse Primers for RT-PCR

Name of gene	5' sequence	3' sequence	Accession no.
SCP1	5' cgccgtcattactcagatcagcaagg 3'	5' gcagtgaacagcacacattcaagagct 3'	AY028804
GAPDH	5' tccaccacctgtgttgctgta 3'	5' accacagtccatgccatcac 3'	NM_008084
ngn1	5' catctctgatctcgactgctccagcag 3'	5' gggtcagagagtgggtgatgccacagtg 3'	NM_010896
beta-tubulin	5' tgccctcacccaagggtctctgacactgtgg 3'	5' ctgaaacagctcctggatggcagtgctg 3'	NM_023716
stra13	5' ctgtggccatggagggaacagtggcttc 3'	5' agaagtcaggagcagctgaggagcac 3'	NM_016665
GAD1	5' gcaaccgcaggcacgactgtttacggag 3'	5' agatgaccatccggaagaagtggccttgt 3'	NM_008077
nrsf	5' ccatcgctgcgaaacctcccaggtaga 3'	5' agccaactcagctggactctctccagcttc 3'	NM_011263

Human Primers for ChIP assay

Name of gene	5' sequence	3' sequence	Accession no.
GAD1 promoter chr2q31	5' tgcggtttatattatcctgcacgccgggag 3'	5' caccggttcgagtcgccggagaggatc 3'	NT005403
GAD1 3' gene chr2q31	5' ggagccctatgcagggttaagggaataa 3'	5' gggctttgattttggagccacctgtg 3'	NT005403
GRIN 2A promoter chr16	5' aactatttctgggtcactccttagacac 3'	5' gctgggaggaatgctttctaagcattg 3'	NT010393
SCN2 promoter chr.2q23	5' ctggataagtactgaagagtgggctttg 3'	5' cagacgacaagttacatgcaacatg 3'	NT005403

10/552298

A

SCP1/UL11F (chr2q35)	(441)	SRKICVTHLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP2/OS4 (chr12q13)	(1160)	GRICVTHLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP3/HTA22 (chr3q22)	(1169)	CRKCVTHLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
PCP1	(1280)	SRKILVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP1/UL11F (chr2q35)	(1131)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP2/OS4 (chr12q13)	(1171)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP3/HTA22 (chr3q22)	(1231)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
PCP1	(1230)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP1/UL11F (chr2q35)	(1184)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP2/OS4 (chr12q13)	(1193)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
SCP3/HTA22 (chr3q22)	(1241)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH
PCP1	(1280)	DEPCVHCLDITLVHCLPKVTHQNP	-	QIPVHICCTVHGVVTKRPH

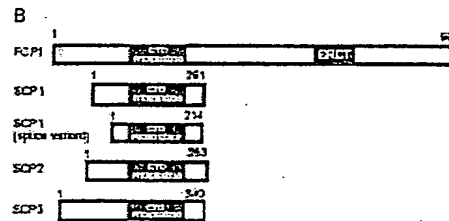


FIG. 1

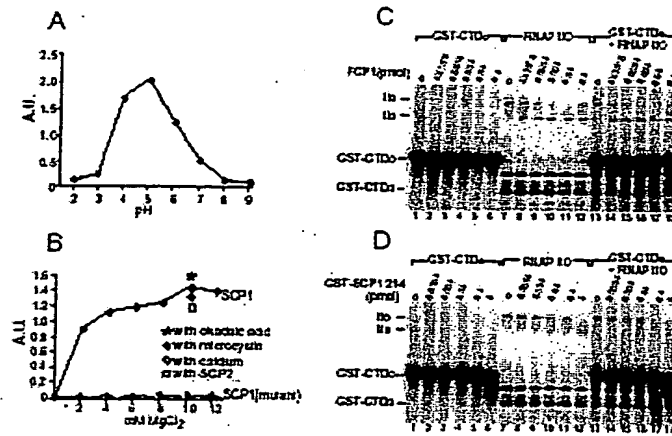


FIG. 2

10/552298

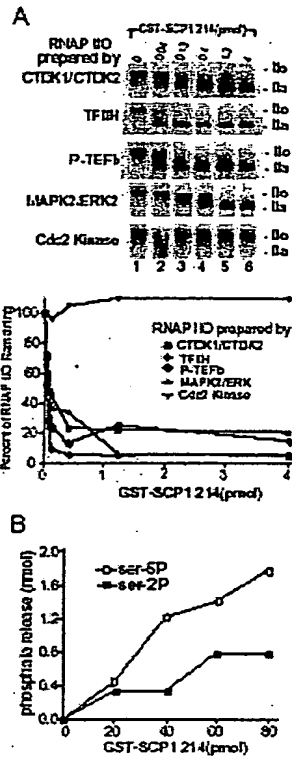


FIG. 3

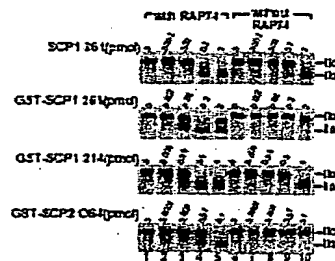


FIG. 4

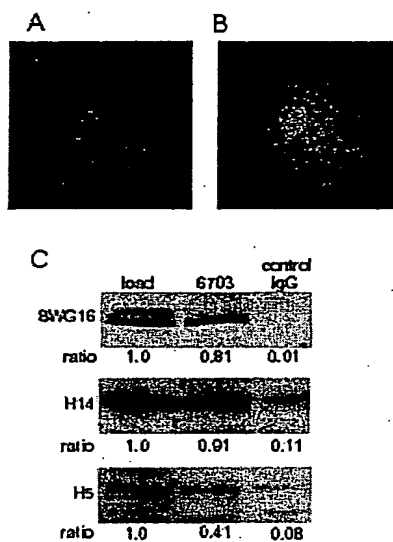


FIG. 5

80/552298

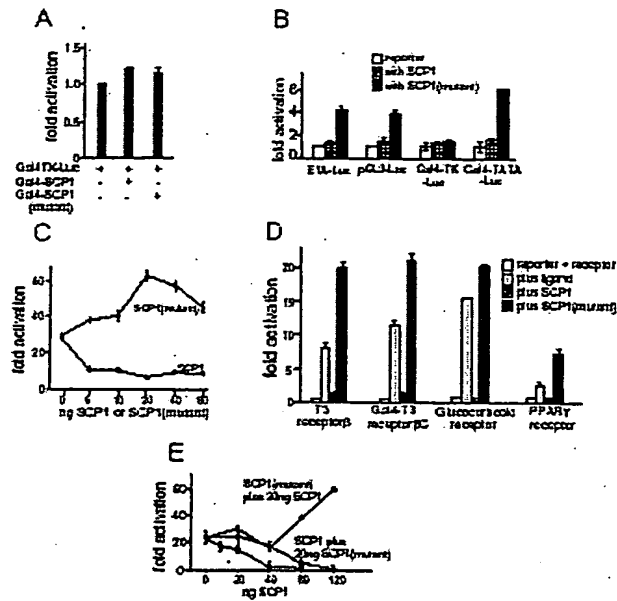


FIG. 6

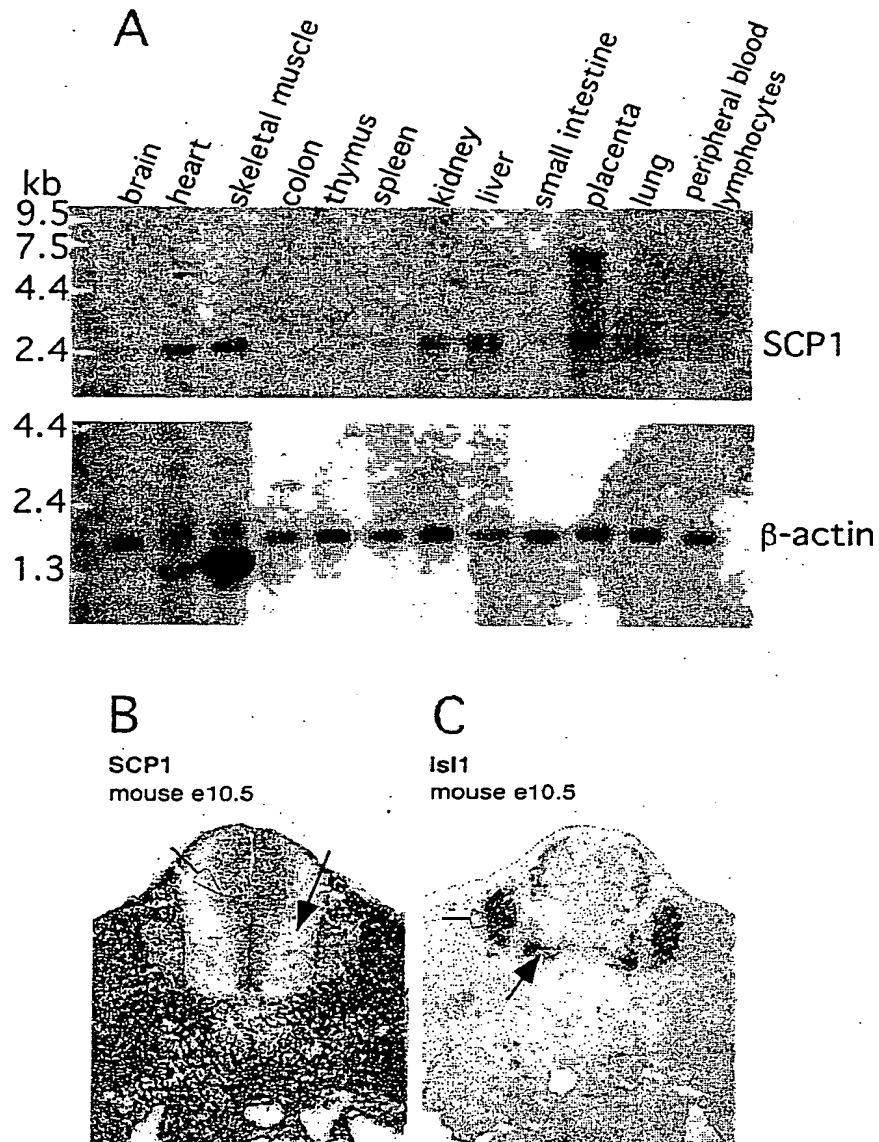


Figure 7

10/552298

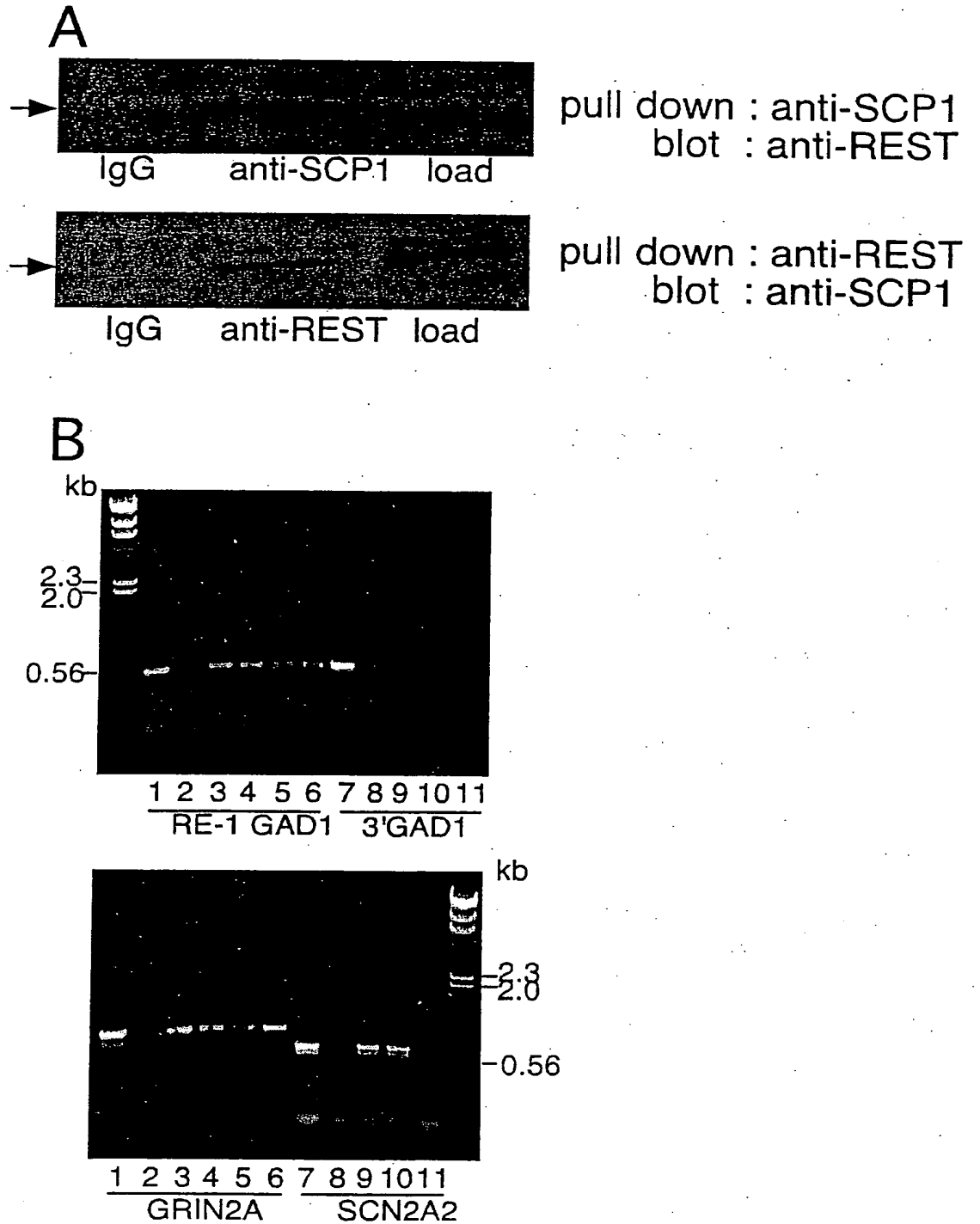
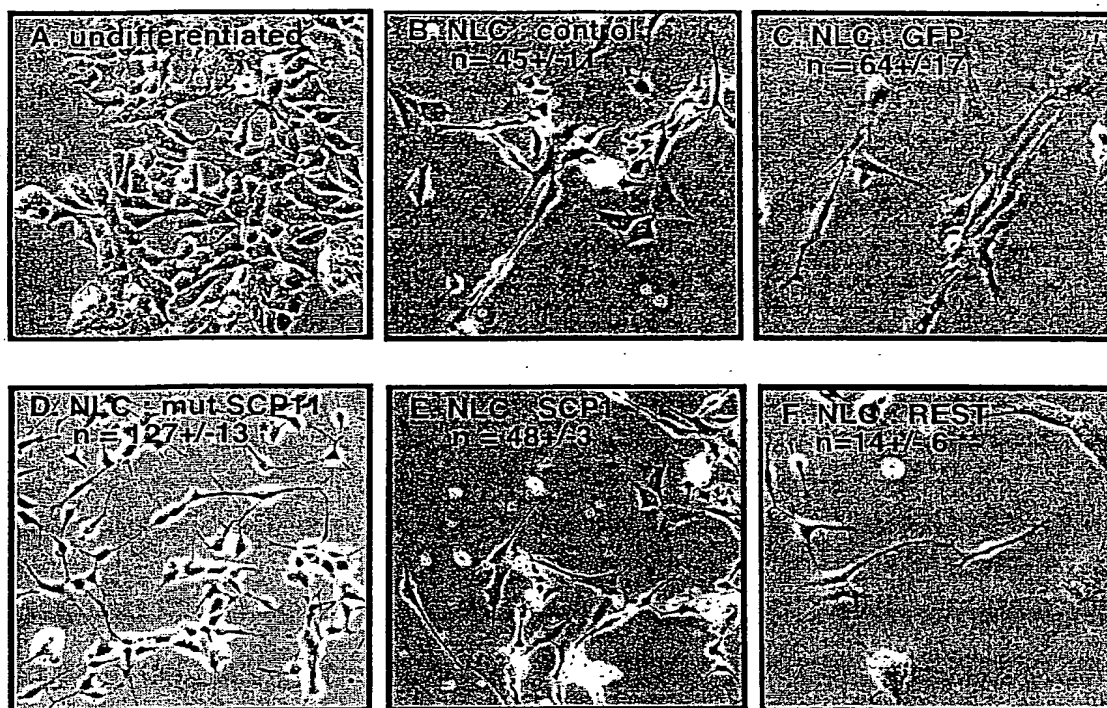


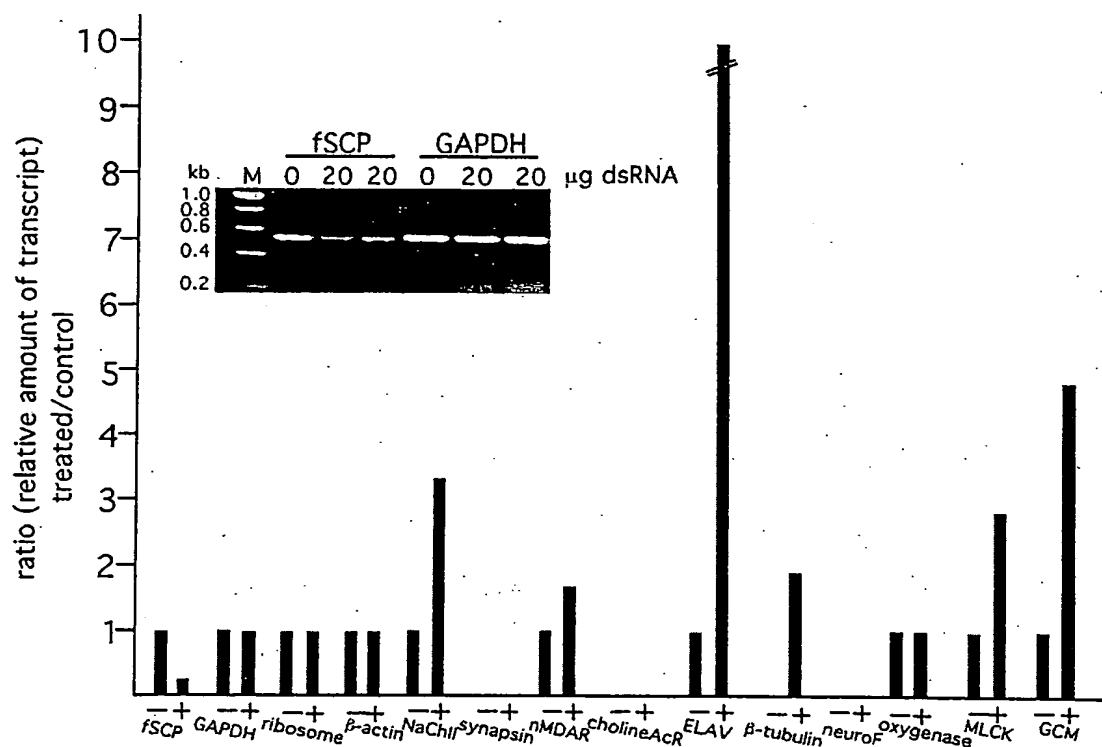
Figure 8

10/552298



* $p = 0.001$ compared to wildtype
** $p = 0.008$ compared to wildtype

Figure 9



gene		average Ct	std deviation
fSCP	control	21.1	0.18
fSCP	knockdown	23.3	0.22
GAPDH	control	17	0.1
GAPDH	knockdown	17	0.19
ribosome	control	25	0.14
ribosome	knockdown	25	0.15
β-actin	control	15	0.1
β-actin	knockdown	15	0.01
NaChl	control	25.1	0.22
NaChl	knockdown	23.8	0.2
synapsin	control	29.5	0.26
synapsin	knockdown	29.5	0.4
nMDAR	control	15.8	0.5
nMDAR	knockdown	15.1	0.16
cholineAcR	control	29.4	0.76
cholineAcR	knockdown	31.2	0.96
ELAV	control	26.8	0.19
ELAV	knockdown	22	0.69
β-tubulin	control	27.9	0.67
β-tubulin	knockdown	25.1	0.35
neuroF	control	30.2	0.83
neuroF	knockdown	30.1	0.55
oxygenase	control	23	0.46
oxygenase	knockdown	23	2.16
MLCK	control	17.8	0.21
MLCK	knockdown	16.5	0.68
GCM	control	23	0.42
GCM	knockdown	21	1.16

Figure 10

<110> THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
 GILL, Gordon N.
 YEO, Michele
 LIN, Patrick S.
 DAHMUS, Michael E.

<120> PHOSPHATASE REGULATION OF NUCLEIC ACID TRANSCRIPTION

<130> UCSD1870WO

<140> PCT/US2004/010218
 <141> 2004-04-01

<150> US 60/459,786
 <151> 2003-04-01

<160> 67

<170> PatentIn version 3.3

<210> 1
 <211> 783
 <212> DNA
 <213> Homo sapiens

<400> 1
 atggacagct cggccgtcat tactcagatc agcaaggagg aggctcgggg cccgctgcgg 60
 ggcaaagggtg accagaagtc agcagcttcc cagaagcccc gaagccgggg catcctccac 120
 tcaactcttct gctgtgtctg cgggatgat ggggaggccc tgcctgctca cagcggggcg 180
 cccctgcttg tggaggagaa tggcgccatc cctaagacc cagtccaata cctgctccct 240
 gaggccaagg cccaggactc agacaagatc tgcgtgggtca tgcacctgga cgagaccctg 300
 gtgcacagct ccttcaagcc agtgaacaac gcggacttca tcatccctgt ggagattgat 360
 ggggtgggtcc accaggtcta cgtgttgaag cgtcctcatg tggatgagtt cctgcagcga 420
 atgggcgagc tctttgaatg tgtgctgttc actgctagcc tcgccaagta cgcagaccca 480
 gtagctgacc tgctggacaa atggggggcc ttccggggcc ggctgtttcg agagtcctgc 540
 gtcttccacc gggggaacta cgtgaaggac ctgagccggt tgggtcgaga cctgcggcgg 600
 gtgctcatcc tggacaattc acctgcctcc tatgtcttcc atccagacaa tgctgtaccg 660
 gtggcctcgt ggtttgacaa catgagtgac acagagctcc acgacctcct ccccttcttc 720
 gagcaactca gccgtgtgga cgacgtgtac tcagtgtcga ggcagccacg gccagggagc 780
 tag 783

<210> 2
 <211> 260
 <212> PRT
 <213> Homo sapiens

<400> 2

Met Asp Ser Ser Ala Val Ile Thr Gln Ile Ser Lys Glu Glu Ala Arg
 1 5 10 15

Gly Pro Leu Arg Gly Lys Gly Asp Gln Lys Ser Ala Ala Ser Gln Lys
 20 25 30

Pro Arg Ser Arg Gly Ile Leu His Ser Leu Phe Cys Cys Val Cys Arg
 35 40 45

Asp Asp Gly Glu Ala Leu Pro Ala His Ser Gly Ala Pro Leu Leu Val
 50 55 60

Glu Glu Asn Gly Ala Ile Pro Lys Thr Pro Val Gln Tyr Leu Leu Pro
 65 70 75 80

Glu Ala Lys Ala Gln Asp Ser Asp Lys Ile Cys Val Val Ile Asp Leu
 85 90 95

Asp Glu Thr Leu Val His Ser Ser Phe Lys Pro Val Asn Asn Ala Asp
 100 105 110

Phe Ile Ile Pro Val Glu Ile Asp Gly Val Val His Gln Val Tyr Val
 115 120 125

Leu Lys Arg Pro His Val Asp Glu Phe Leu Gln Arg Met Gly Glu Leu
 130 135 140

Phe Glu Cys Val Leu Phe Thr Ala Ser Leu Ala Lys Tyr Ala Asp Pro
 145 150 155 160

Val Ala Asp Leu Leu Asp Lys Trp Gly Ala Phe Arg Ala Arg Leu Phe
 165 170 175

Arg Glu Ser Cys Val Phe His Arg Gly Asn Tyr Val Lys Asp Leu Ser
 180 185 190

Arg Leu Gly Arg Asp Leu Arg Arg Val Leu Ile Leu Asp Asn Ser Pro
 195 200 205

Ala Ser Tyr Val Phe His Pro Asp Asn Ala Val Pro Val Ala Ser Trp
 210 215 220

Phe Asp Asn Met Ser Asp Thr Glu Leu His Asp Leu Leu Pro Phe Phe
 225 230 235 240

Val Leu Thr Lys Gln Gly Leu Val Ser Lys Ser Ser Pro Lys Lys Pro
20 25 30

Arg Gly Arg Asn Ile Phe Lys Ala Leu Phe Cys Cys Phe Arg Ala Gln
 35 40 45
 His Val Gly Gln Ser Ser Ser Ser Thr Glu Leu Ala Ala Tyr Lys Glu
 50 55 60
 Glu Ala Asn Thr Ile Ala Lys Ser Asp Leu Leu Gln Cys Leu Gln Tyr
 65 70 75 80
 Gln Phe Tyr Gln Ile Pro Gly Thr Cys Leu Leu Pro Glu Val Thr Glu
 85 90 95
 Glu Asp Gln Gly Arg Ile Cys Val Val Ile Asp Leu Asp Glu Thr Leu
 100 105 110
 Val His Ser Ser Phe Lys Pro Ile Asn Asn Ala Asp Phe Ile Val Pro
 115 120 125
 Ile Glu Ile Glu Gly Thr Thr His Gln Val Tyr Val Leu Lys Arg Pro
 130 135 140
 Tyr Val Asp Glu Phe Leu Arg Arg Met Gly Glu Leu Phe Glu Cys Val
 145 150 155 160
 Leu Phe Thr Ala Ser Leu Ala Lys Tyr Ala Asp Pro Val Thr Asp Leu
 165 170 175
 Leu Asp Arg Cys Gly Val Phe Arg Ala Arg Leu Phe Arg Glu Ser Cys
 180 185 190
 Val Phe His Gln Gly Cys Tyr Val Lys Asp Leu Ser Arg Leu Gly Arg
 195 200 205
 Asp Leu Arg Lys Thr Leu Ile Leu Asp Asn Ser Pro Ala Ser Tyr Ile
 210 215 220
 Phe His Pro Glu Asn Ala Val Pro Val Gln Ser Trp Phe Asp Asp Met
 225 230 235 240
 Ala Asp Thr Glu Leu Leu Asn Leu Ile Pro Ile Phe Glu Glu Leu Ser
 245 250 255
 Gly Ala Glu Asp Val Tyr Thr Ser Leu Gly Ala Ala Ala Gly Pro Leu
 260 265 270

Ala Cys Pro Ala Ser Lys Arg Arg Pro Ser Gln
 275 280

<210> 5
 <211> 798
 <212> DNA
 <213> Homo sapiens

<400> 5
 atggacggcc cggccatcat caccaggtg accaacccca aggaggacga gggccggttg 60
 ccgggcgcgg gcgagaaagc ctcccagtg aacgtcagct taaagaagca gaggagccgc 120
 agcatcctta gtccttctt ctgctgcttc cgtgattaca atgtggaggc ccctccaccc 180
 agcagcccca gtgtgcttcc gccactggtg gaggagaatg gtgggcttca gaagccacca 240
 gctaagtacc ttcttccaga ggtgacggtg cttgactatg gaaagaaatg tgtggtcatt 300
 gatttagatg aaacattggt gcacagttcg tttaaagccta ttagtaatgc tgattttatt 360
 gttccggttg aaatcgatgg aactatacat caggtgtatg tgctgaagcg gccacatgtg 420
 gacgagttcc tccagaggat ggggcagctt tttgaatgtg tgctctttac tgccagcttg 480
 gccaaagtatg cagaccctgt ggctgacctc ctagaccgct ggggtgtgtt ccgggcccgg 540
 ctcttcagag aatcatgtgt tttcatcgt gggaactacg tgaaggacct gagtcgcctt 600
 gggcgggagc tgagcaaagt gatcattgtt gacaattccc ctgcctcata catcttccat 660
 cctgagaatg cagtgcctgt gcagtcctgg ttogatgaca tgacggacac ggagctgctg 720
 gacctcatcc ccttctttga gggcctgagc cgggaggacg acgtgtacag catgctgcac 780
 agactctgca ataggtag 798

<210> 6
 <211> 265
 <212> PRT
 <213> Homo sapiens

<400> 6

Met Asp Gly Pro Ala Ile Ile Thr Gln Val Thr Asn Pro Lys Glu Asp
 1 5 10 15

Glu Gly Arg Leu Pro Gly Ala Gly Glu Lys Ala Ser Gln Cys Asn Val
 20 25 30

Ser Leu Lys Lys Gln Arg Ser Arg Ser Ile Leu Ser Ser Phe Phe Cys
 35 40 45

Cys Phe Arg Asp Tyr Asn Val Glu Ala Pro Pro Pro Ser Ser Pro Ser
 50 55 60

Val Leu Pro Pro Leu Val Glu Glu Asn Gly Gly Leu Gln Lys Pro Pro
65 70 75 80

Ala Lys Tyr Leu Leu Pro Glu Val Thr Val Leu Asp Tyr Gly Lys Lys
85 90 95

Cys Val Val Ile Asp Leu Asp Glu Thr Leu Val His Ser Ser Phe Lys
100 105 110

Pro Ile Ser Asn Ala Asp Phe Ile Val Pro Val Glu Ile Asp Gly Thr
115 120 125

Ile His Gln Val Tyr Val Leu Lys Arg Pro His Val Asp Glu Phe Leu
130 135 140

Gln Arg Met Gly Gln Leu Phe Glu Cys Val Leu Phe Thr Ala Ser Leu
145 150 155 160

Ala Lys Tyr Ala Asp Pro Val Ala Asp Leu Leu Asp Arg Trp Gly Val
165 170 175

Phe Arg Ala Arg Leu Phe Arg Glu Ser Cys Val Phe His Arg Gly Asn
180 185 190

Tyr Val Lys Asp Leu Ser Arg Leu Gly Arg Glu Leu Ser Lys Val Ile
195 200 205

Ile Val Asp Asn Ser Pro Ala Ser Tyr Ile Phe His Pro Glu Asn Ala
210 215 220

Val Pro Val Gln Ser Trp Phe Asp Asp Met Thr Asp Thr Glu Leu Leu
225 230 235 240

Asp Leu Ile Pro Phe Phe Glu Gly Leu Ser Arg Glu Asp Asp Val Tyr
245 250 255

Ser Met Leu His Arg Leu Cys Asn Arg
260 265

<210> 7
<211> 642
<212> DNA
<213> Homo sapiens

<400> 7
atgatgggga ggccctgcct gctcacagcg gggcgccct gcttgtggag gagaatggcg

```

ccatccctaa ggcagacccc agtccaatac ctgctccctg aggccaaggc ccaggactca      120
gacaagatct gcgtgggtcat cgacctggac gagaccctgg tgcacagctc cttcaagcca      180
gtgaacaacg cggacttcat catccctgtg gagattgatg ggggtggcca ccagggtctac      240
gtgttgaagc gtccctcacgt ggatgagttc ctgcagcgaa tgggcgagct ctttgaatgt      300
gtgctgttca ctgctagcct cgccaagtac gcagacccag tagctgacct gctggacaaa      360
tggggggcct tccggggccg gctgtttcga gagtccctgcg tcttccaccg ggggaactac      420
gtgaaggacc tgagccgggtt gggtcgagac ctgcggcggg tgctcctcct ggacaattca      480
cctgcctcct atgtcttcca tccagacaat gctgtaccgg tggcctcgtg gtttgacaac      540
atgagtgaca cagagctcca cgacctctc cccttcttcg agcaactcag ccgtgtggac      600
gacgtgtact cagtgtcag gcagccacgg ccaggagact ag                          642

```

```

<210> 8
<211> 213
<212> PRT
<213> Homo sapiens

```

```

<400> 8

```

```

Met Met Gly Arg Pro Cys Leu Leu Thr Ala Gly Arg Pro Cys Leu Trp
1           5           10           15

```

```

Arg Arg Met Ala Pro Ser Leu Arg Gln Thr Pro Val Gln Tyr Leu Leu
          20           25           30

```

```

Pro Glu Ala Lys Ala Gln Asp Ser Asp Lys Ile Cys Val Val Ile Asp
          35           40           45

```

```

Leu Asp Glu Thr Leu Val His Ser Ser Phe Lys Pro Val Asn Asn Ala
          50           55           60

```

```

Asp Phe Ile Ile Pro Val Glu Ile Asp Gly Val Val His Gln Val Tyr
65           70           75           80

```

```

Val Leu Lys Arg Pro His Val Asp Glu Phe Leu Gln Arg Met Gly Glu
          85           90           95

```

```

Leu Phe Glu Cys Val Leu Phe Thr Ala Ser Leu Ala Lys Tyr Ala Asp
          100          105          110

```

```

Pro Val Ala Asp Leu Leu Asp Lys Trp Gly Ala Phe Arg Ala Arg Leu
          115          120          125

```

```

Phe Arg Glu Ser Cys Val Phe His Arg Gly Asn Tyr Val Lys Asp Leu

```

130

135

140

Ser Arg Leu Gly Arg Asp Leu Arg Arg Val Leu Ile Leu Asp Asn Ser
 145 150 155 160

Pro Ala Ser Tyr Val Phe His Pro Asp Asn Ala Val Pro Val Ala Ser
 165 170 175

Trp Phe Asp Asn Met Ser Asp Thr Glu Leu His Asp Leu Leu Pro Phe
 180 185 190

Phe Glu Gln Leu Ser Arg Val Asp Asp Val Tyr Ser Val Leu Arg Gln
 195 200 205

Pro Arg Pro Gly Ser
 210

<210> 9
 <211> 783
 <212> DNA
 <213> Drosophila

<400> 9
 atggacagct cggccgctcat tactcagatc agcaaggagg aggctcgggg cccgctgcgg 60
 ggcaaagggtg accagaagtc agcagcttcc cagaagcccc gaagccgggg catcctccac 120
 tcactcttct gctgtgtctg cgggatgat ggggaggccc tgcctgctca cagcggggcg 180
 cccctgcttg tggaggagaa tggcgccatc cctaagacct cagtccaata cctgctccct 240
 gaggccaagg cccaggactc agacaagatc tgcgtggtca tgarctgaa cgagaccctg 300
 gtgcacagct ccttcaagcc agtgaacaac gcggacttca tcatccctgt ggagattgat 360
 ggggtggtcc accaggtcta cgtgttgaag cgtcctcatg tggatgagtt cctgcagcga 420
 atgggcgagc tctttgaatg tgtgctgttc actgctagcc tcgccaagta cgcagacca 480
 gtagctgacc tgctggacaa atggggggcc ttccggggcc ggctgtttcg agagtcctgc 540
 gtcttccacc gggggaacta cgtgaaggac ctgagccggt tgggtcgaga cctgcggcgg 600
 gtgctcatcc tggacaattc acctgcctcc tatgtcttcc atccagacaa tgctgtaccg 660
 gtggcctcgt ggtttgacaa catgagtac acagagctcc acgacctcct ccccttcttc 720
 gagcaactca gccgtgtgga cgacgtgtac tcagtgtca ggcagccacg gccagggagc 780
 tag 783

<210> 10
 <211> 260
 <212> PRT

<213> Drosophila

<400> 10

Met Asp Ser Ser Ala Val Ile Thr Gln Ile Ser Lys Glu Glu Ala Arg
1 5 10 15

Gly Pro Leu Arg Gly Lys Gly Asp Gln Lys Ser Ala Ala Ser Gln Lys
20 25 30

Pro Arg Ser Arg Gly Ile Leu His Ser Leu Phe Cys Cys Val Cys Arg
35 40 45

Asp Asp Gly Glu Ala Leu Pro Ala His Ser Gly Ala Pro Leu Leu Val
50 55 60

Glu Glu Asn Gly Ala Ile Pro Lys Thr Pro Val Gln Tyr Leu Leu Pro
65 70 75 80

Glu Ala Lys Ala Gln Asp Ser Asp Lys Ile Cys Val Val Ile Glu Leu
85 90 95

Asn Glu Thr Leu Val His Ser Ser Phe Lys Pro Val Asn Asn Ala Asp
100 105 110

Phe Ile Ile Pro Val Glu Ile Asp Gly Val Val His Gln Val Tyr Val
115 120 125

Leu Lys Arg Pro His Val Asp Glu Phe Leu Gln Arg Met Gly Glu Leu
130 135 140

Phe Glu Cys Val Leu Phe Thr Ala Ser Leu Ala Lys Tyr Ala Asp Pro
145 150 155 160

Val Ala Asp Leu Leu Asp Lys Trp Gly Ala Phe Arg Ala Arg Leu Phe
165 170 175

Arg Glu Ser Cys Val Phe His Arg Gly Asn Tyr Val Lys Asp Leu Ser
180 185 190

Arg Leu Gly Arg Asp Leu Arg Arg Val Leu Ile Leu Asp Asn Ser Pro
195 200 205

Ala Ser Tyr Val Phe His Pro Asp Asn Ala Val Pro Val Ala Ser Trp
210 215 220

Phe Asp Asn Met Ser Asp Thr Glu Leu His Asp Leu Leu Pro Phe Phe

Leu Asn Glu Thr Leu Val His Ser Ser Phe Lys Pro Val Asn Asn Ala
50 55 60

Asp Phe Ile Ile Pro Val Glu Ile Asp Gly Val Val His Gln Val Tyr
65 70 75 80

Val Leu Lys Arg Pro His Val Asp Glu Phe Leu Gln Arg Met Gly Glu
85 90 95

Leu Phe Glu Cys Val Leu Phe Thr Ala Ser Leu Ala Lys Tyr Ala Asp
100 105 110

Pro Val Ala Asp Leu Leu Asp Lys Trp Gly Ala Phe Arg Ala Arg Leu
115 120 125

Phe Arg Glu Ser Cys Val Phe His Arg Gly Asn Tyr Val Lys Asp Leu
130 135 140

Ser Arg Leu Gly Arg Asp Leu Arg Arg Val Leu Ile Leu Asp Asn Ser
145 150 155 160

Pro Ala Ser Tyr Val Phe His Pro Asp Asn Ala Val Pro Val Ala Ser
165 170 175

Trp Phe Asp Asn Met Ser Asp Thr Glu Leu His Asp Leu Leu Pro Phe
180 185 190

Phe Glu Gln Leu Ser Arg Val Asp Asp Val Tyr Ser Val Leu Arg Gln
195 200 205

Pro Arg Pro Gly Ser
210

<210> 13
<211> 7020
<212> DNA
<213> Drosophila

<400> 13
ctggagcgcg gcaggaaccc ggcccggccc gcctcccagt ccgcctagcc gcgccggtcc 60
cagaagtggc gaaagccgca gccgagtcca ggacacgccg aagccgttgc ccttttaagg 120
gggagccttg aaacggcgcc tgggttccat gtttgcattc gcctcgcggg aaggaaactc 180
catgttgtaa caaagtttcc tccgcgcccc ctccctcccc ctcccccta gaacctggct 240
ccccccccct ccggagctcg cggggatccc tccctccccc cctcccccct cccccgcgc 300
ccgattccg gccccagccg ggggggaggg cgggcgcccc ggccagagtc cggccggagc 360
ggagcgcgcc cggcccatg gacagctcgg ccgtcattac tcagatcagc aaggaggagg 420

ctcggggccc gctgcggggc aaaggtaccg gggctgcggg gagggggccg aagccggggc	480
gccgtgggag gagagaaggg gccgggatct tccccagggg agccgccgcc gccgccccgg	540
gcggccgcct tagctgtgcc cgaagctccc agcccagagag ggagcagggg gagagtttga	600
actcagagga ggctcagaga cgcggggcgg ggctggcgc ctttggggcg ctctgtccg	660
ctcgaggtga ggaaactgag gcaggaatag agaggggaact ccttcggggg tttcctggca	720
ggcattgcgt ggtgcatggg cccccccca ccattggcgc caatggggct gtgagatggg	780
ggagctgagg agggcgccca tggggcaccc gctgagactc cggcccacc cccaccccca	840
ccccccggg ctgcggtccg gtagggctctt gggagggggc gccgaggtga cagcaggctg	900
gggaggcttg gagggatctc ccgccaacac acagctacgt tccccacaaa cttcgcgtca	960
cgcgtggagg cgcgcacccc ctgcggaggc cagagaggac ggccggcact tccaagagtc	1020
gcttggcgcc cgcggggaga gtcgtgcgcc tagtgggcac gcaccacccc gcaaagcctc	1080
gccgccccga cgaggctgcg tccccagcg tggctgggcc ggggtggggg ggtctgtctt	1140
ctccttttcc ccgtgtggac ctcaggatct ggacgtgcc ccagggtctg cccaccctcg	1200
cctgggtctg gctgccccgg aactgagggc aagtggaag ggctagttgc agggggccgg	1260
aggggggtgg ggtgggaggg gtatctgtca atcaggctgc tgggtccag gtcggaggtc	1320
tgggcggggc agggcaaaca gatggccact ggacactggc ccaggccgc gggactgcac	1380
ccctgcctct gggcccagcc gcagtgagga cttcgtaccc acgggggtgg agaggatgga	1440
gggagggcag ggggtgactg ccctgggtcc caggccctgg ctgtcctgag caggggtgct	1500
caggtaaggt ggggtcagga ggcaccgcaa tggggctgat cagcagcagt catggaggct	1560
gtgagaggca gggagagagc accccaggac ctcttctcc aggccacgca ctccctatgt	1620
gggcgcctta atacctgcta gacctatttg tctgggagct gcaggagcct tggagttgat	1680
tgtggagccc tgacaggggc gtttcagaga aagtcaggag ctgccttcgt gtgtctggat	1740
gaaggggcca cggcaagatc ctctggccc aggggttcac acctgggcac acatgcagga	1800
ttctgcaggc cagtgtgcac cgagcctcca acttgtgcct ccctacttca ggtgaccaga	1860
agtcagcagc tccccagaag ccccggaagc ggggcatcct ccactcactc ttctgctgtg	1920
tctgccggga tgatggggag gccctgcctg ctcacagcgg ggcgcccctg cttgtggagg	1980
agaatggcgc catccctaag gtgcgtgggg gccaggtggg gccacggggg cacctggact	2040
cagtcttcag ggcttttaggg gaaggggctc ctgactgagc ttttcaggat ggacttgag	2100
acctgaaagt gcagagtagg aggggtggcag cctccctgc caggccctgc ccactgtggg	2160
gaaactgaat tctccctcat aagtggaagc tttttctac cttggttttt agagaggtct	2220

caaagagcca agaggcctac ccaagcccta gagctggcag gggcaaagct gggaaggggg	2280
aagtatctgt tccctggggcc tgggggttcct ctggagacgg ctagggggag aagcctgcgt	2340
gggaggaagg accaggcccc gagagaggca cccagccag ccccgccctc cctacagcag	2400
accccatgtcc aatacctgct ccctgaggcc aaggccagg actcagacaa gatctgcgtg	2460
gtcatcgacc tggacgagac cctgggtgcac agctccttca aggtggggcc tgctcaacag	2520
ccctcagccc ggggtctcggg gggcatcccc caccctggcc tgggaggagg gtgtgtgctg	2580
gaccccatgc cctggggctc ctccctcaac tccagcagct cttttccccc cacagccagt	2640
gaacaacgcg gacttcatca tccctgtgga gattgatggg gtgggtccacc aggtgagggc	2700
caggaagagg cagtgggtggg cttggcatct gcctccagac ctagggtctt tcccaccaat	2760
ccggagcgcc tcggatggga attggataca tgtggaatgt cagaggccca gagagggtgt	2820
gagacttgtc ccaaagtcac acagaacctc aagggttgt gctgactcca agcctgcaga	2880
gtgggtcct cctctaggct ccccgctgct gtgctccctc gcccaccct gccgggacc	2940
cagttcaagt aattcaggat aggttggtgt ctgtccagcc tgttctccat tacttggtc	3000
ggggaccggt gccctgcagc cttgggggtga gggggctgcc cctggattcc tgcactaggc	3060
tgaggttgag gcaggggaag ggattgggaa ttagggacct cgtgaggtag gactggccag	3120
tggagtggaa gttttgatcg ttttctggcg gggggtgggt acagtttccc cagcagtgg	3180
cagggtagct ggccaagcgg agcctgcggg cccagtctcc ttctgtgcg cctctgcctc	3240
cctggcccat gccctgccag ccctcgcca ccccccact gcccactgg cccgcagccc	3300
cctcactggc ccgccccca ggtctacgtg ttgaagcgtc ctcatgtgga tgagtccctg	3360
cagcgaatgg gcgagctctt tgaatgtgtg ctgttcactg ctagcctcgc caaggtgagc	3420
cccacagggg tcccggggca accctgccct cctacctacc tccgcagtc agcccagtga	3480
acctgcgggc cccaggatga cccacctcct gctcccagta cgcagaccca gtagctgacc	3540
tgctggacaa atggggggcc ttccgggccc ggctgtttcg agagtcctgc gtcttcacc	3600
gggggaacta cgtgaaggac ctgagccggt tgggtcgaga cctgcggcgg gtgctcatcc	3660
tggacaattc acctgcctcc tatgtcttcc atccagacaa tgctgtgagt gcgggctgga	3720
ctgggactgg gacaggagct gagaccagg aagggtcag tccattcagg ccaccttggc	3780
ctcttggaac cccagttggg ggggtgggtgc cctcccagtc cttcctgcat tcattgcctg	3840
tgctgcccgc cactccct catccacctg ccctgtagcc atatggtctt tccccctgc	3900
acaaagcaga gcatctgcca tgcacagggg cccccacagg gcaacggagt ttggaaagtt	3960
tcaatttttc gaattgccag ttgtgacct ctgatggccc acagaattaa tttagtgggt	4020
tctgattggg aattttaaca aaatgaaata gaatagaaaa tatccggtcg ggtgcagtgg	4080

ctcatgcctg taatcccagc actttgggaa gctgaggtgg gcaggtagct gagcccagta	4140
gttcaagacc agcctcggca acatagtgaa accttatgtc tacaaaaaat aaaaaaacta	4200
gccaggcgtg gtggcgcatg cctggagtcc cggctatgca gaaggctgag gtaggagtat	4260
cgcttgagcc ctggaggcag aggctgtggt gagccaagat tgtgccactg cactctagcc	4320
tgggcaacag agcaagaccc tgcctcaaaa aaaaaaaaaa gtatccaagt gcttcgcaca	4380
gataaggtta ggaattgtga agcttttgca ttgttacgtt ataaatgtgt tttcctgggg	4440
attgctgtca aaaaagtttg aacactgtgg gtgaggggtt ttcagaaact gcatgatctg	4500
agtagtggct acatagggct ggcttgaaa ttctgcaccc aggaccacct gccccctca	4560
tcttctaca ccacttccc caggtaccgg tggcctcgtg gtttgacaac atgagtgaca	4620
cagagctcca cgacctctc cccttcttcg agcaactcag ccgtgtggac gacgtgtact	4680
cagtgtcag gcagccacgg ccaggagct agtgaggggtg atggggccag gacctgcccc	4740
tgaccaatga taccacacc tcctcccagg aagactgcc aggcccttgt taggaaaacc	4800
catgggccgc cgccacactc agtgccatgg ggaagcgggc gtctcccca ccagccccac	4860
caggcggtgt aggggcagca ggctgcactg aggaccgtga gctccaggcc ccgtgtcagt	4920
gccttcaaac ctctccctt attctcaggg gacctggggg gcctgcctg ctgctccctt	4980
tttctgtctc tgtccatgct gccatgtttc tctgtgcc aattggggcc cttggccctt	5040
tccggttctg ctctcctggg gcagggttcc tgccttgga cccagctctg ggaacgggtg	5100
acatcaagtg ccttgcatag agccccctt tccccgcca gctttcccag gggcacagct	5160
ctaggctggg aggggagaa cagccccctc ccctgcccc cctcctcctt tgggactgag	5220
agggccccta ccaacctttg cctctgcctt ggaggagggg gaggtctgtt accactgggg	5280
aaggcagcag gagtctgtcc ttcaggcccc acagtgcagc ttctccaggg ccgacagctg	5340
agggtgtctc cctgcatcat ccaagcaatg acctcagact tctgccttaa ccagccccgg	5400
ggcttggtc cccagctct gagcgtggg gcataggcag gacccccctt gtggtgccat	5460
ataaatatgt acatgtgtat atagattttt aggggaagga gagagggaag ggtcagggtg	5520
gagacacccc tcccttgccc ctttctcggg ccagaaagt ggggggaggg agggaaagga	5580
tttttacatt ttttaactg ctattttctg aatggaacaa gctgggcca ggggccagg	5640
ccctgtctc tgtccctcac acccctttgc tccgttcatt cattcaaaaa aacatttctt	5700
gagcaccttc tgtgccagc atatgctagg ccaccagct aagtgtgtgt ggggggtctc	5760
tacgccagct catcagtgc tccttgcca tccttcaccg gtgcctttgg gggatctgta	5820
ggaggtggga ccttctgtgg ggtttgggga tctccaggaa gcccagccaa gctgtccctt	5880

tccccctgtgc caacccatct cctacagccc cctgcctgat cccctgctgg ctgggggagcag 5940
 ctcccaggat atcctgcctt ccaactgttt ctgaagcccc tctcctaac atggcgattc 6000
 cggaggtcaa ggccttgggc tctccccagg gtctaacggt taaggggacc cacataccag 6060
 tgccaagggg gatgtcaagt ggtgatgtcg ttgtgctccc ctccccaga gcgggtgggc 6120
 ggggggtgaa tatggttggc ctgcatcagg tggccttccc atttaagtgc cttctctgtg 6180
 actgagagcc ctagtgtgat gagaactaaa gagaaagcca gaccctatc ctgcttctgt 6240
 gggtatttgcg ggggacttca gcaagtgggg tgtgtgcctt gcacctgcgg ctgccgtggg 6300
 cccccccccc gcttcagcac acctagaggg ctgttgggtg agggaggggc tgcccggccc 6360
 tcgacacttc aggtgggaag ggcagcgtca gagcacaat ttgagcctcc aggctgtgct 6420
 cgtctacgtc ttcccgcctc gggatatgtg tctgcaaaat ggagatgtgc cctatttgga 6480
 ggactaatta agtgcctgga cacagacgac aggatactag tagctggaaa gcaaaattcg 6540
 aaggcctggg taggggcagt cctggaatgc ggcgggggag ggggcgtggc ctctgccctg 6600
 gagcagaggg gcggggcttg tgcggctccg aaggcagagg cggggagcgg ggcgaggctc 6660
 tgggtggagg ctccagcggc agaacttgtt ggcttgggtg cggcgggctc cggcgcttg 6720
 ctctgccggg cggcctgggt ggggcccggc cgggggctcg gcccccccg cccctctgcg 6780
 gcctctgagc agccattggc cgcgcccccg cccacttcc cgccccgcc cgcgctccgg 6840
 aggcacttcc ttgcgaaac cgcgcggccc caggcgccgg caggaaatgc cctcccgccc 6900
 tccccagcca gcctttgctt gcttcccacg ccagccgcta gaggcctccc tgcctctgcg 6960
 gacgcaggaa ctccccgggg gctggaaaga tggggcccac ctactcacc cctttcccg 7020

<210> 14
 <211> 4833
 <212> DNA
 <213> Homo sapiens

<400> 14
 gccatttcct cctcttgttt tctactccga ttctccatgt tggacccaaa ctgaggagcc 60
 cggagctgcc gctgggggat cggggccggg ggcacccggg ggagccgctg cccgggcccgc 120
 ccgccctttg tacaggccgc ctcccttccc ggtccgggga ggaaacgaga ggggggatgt 180
 gaacagctgt ggaagtcgga gtctcgggag ccggagcggg cccccgccca ggccccccag 240
 ccagcccag cccgcgcgcc cgcccgctct cccgtccagc cagcccgggc ccgcgggatt 300
 gttagatgga acacggctcc atcatcacc aggcgcggag ggaagacgcc ctggtgtc 360
 ccaagcaagg cctggtctcc aagtcctctc ctaagaagcc tcgtggacgt aacatcttca 420
 aggccctttt ctgctgtttt cgcgcccagc atgttggcca gtcaagttcc tccactgagc 480

tcgctgcgta taaggaggaa gcaaacacca ttgctaagtc ggatctgctc cagtgtctcc	540
agtaaccagtt ctaccagatc ccagggacct gcctgctccc agaggtgaca gaggaagatc	600
aaggaaggat ctgtgtggtc attgacctcg atgaaaccct tgtgcatagc tcctttaagc	660
caatcaacaa tgctgacttc atagtgccta tagagattga ggggaccact caccaggtgt	720
atgtgctcaa gaggccttat gtggatgagt tcctgagacg catgggggaa ctctttgaat	780
gtgtttctct cactgccagc ctggccaagt atgccgacct tgtgacagac ctgctggacc	840
ggtgtggggt gttccgggcc cgcctattcc gtgagtcttg cgtgttcac cagggctgct	900
acgtcaagga cctcagccgc ctggggaggg acctgagaaa gaccctcatc ctggacaact	960
cgcctgcttc ttacatattc caccocgaga atgcagtgcc tgtgcagtc tggtttgatg	1020
acatggcaga cactgagttg ctgaacctga tcccaatctt tgaggagctg agcggagcag	1080
aggacgtcta caccagcctt ggggcagctg cgggccctt agcctgccct gcttccaagc	1140
gacggccatc ccagtagggg actttccac actgtgcctt taogatcagc gtgacagagt	1200
agaagctgga gtgcctcacc acacggcccg gaaacagcgg gaagtaactg gaaagagctt	1260
taggacagct tagatgccga gtgggcgaat gccagaccaa tgataccag agctacctgc	1320
cgccaaacttg ttgagatgtg tgtttgactg tgagagagtg tgtgtttgtg tgtgtgtttt	1380
gccatgaact gtggccccag tgtatagtgt ttcagtgggg gagaagctga aagaccaaga	1440
ctcttcccaa gttagcttgt ctctctcct gtcaccctaa gagccactga gttgtgtagg	1500
gatgaaract attgaagact ccattgccaa accatggcct ttcctcagtg ttgtaaggcc	1560
tatgccaaagg ataaaggaag ggtatgcctt tgggtactcc aggcatacac ctttctgaaa	1620
tccttctcca gccagctgct gcagacaaaa gatcacattt ctgggaagat gagaacttgt	1680
ttccagacca gcatccagtg gccatcaggt cttgtggccc aaaggctatg cttgcctccg	1740
gctgagtgcc tgggataggc cttttctatg tctccccaag gctgggggtgc tgagcctgcc	1800
ttcctcacca cctagccata gtctcaaacc tgtggggaag gaggttttct ccctgcccg	1860
gaagaggaca gataactgat ttccgttctt ttgactgtgt tttaaaattc tctttctaaa	1920
cacagagtgt tgggcctggg ttgtttctga caaagttaca gtccctgggc tgtaatgaat	1980
gtcggcggcg ctgggggtgc agggaaaaga caaatcctca aagcgtggac gtgtgtcccc	2040
atggcttgtg gatcagctaa gctcgggatc atttccataa gtctgctttt cagggattct	2100
ctgctgggtg tggtgcaagg acttctgttc caaaggctgg gaaaaactaa gctgtcccag	2160
cccccccat ttcttgggca gggctctttt cctgttgtgt ctccccccag ggctgtcct	2220
gtaccgagct ctgtctgttc cagcctacat ccttcctggg tgttgctttt cctcttaagg	2280
gcctcagaac tcttgctctt cctgggggtga gggggaatga gtgttcttga catgtgacag	2340

cctaatagcgc atgcttttctg cctctggtaa caggagttag tgagcccctc agacctgcac	2400
tctgggtgtc tcttgcttac aaaggttctt aatagtgaat gctttaaaat taaagtcac	2460
acgaaatgga agttttccca gggtggaata taagaggaag tgctgctgta attgggagca	2520
caaggggcct cccaaaaagg agccccacct cagcatcact gccttaatcg tggcctccct	2580
gggggtgggtg ggggttctctc ctccctccct cctcctcct ggggtgggag ggcgtcctg	2640
ttcccatctc tgtgttccct ggaggcaggt atcacaagc atttgtagat tgcttttagt	2700
gcaggagacac caccactca ggactcttcc ccatcatccc ttccattgcc acaccctaga	2760
tccagcctca ggaactaaca agttktgaga aaagcaggtg gtagagcagc agcttcgtgc	2820
tctcagcggg ggctggctgg catttttctc tagcgttgtg gtgccacctt ccttcttgt	2880
cccaagggtta taaggccttg tctttctctt tggaatcata aagtggaaac gagtccccag	2940
aactcatgtg ghcatttccg acagcatcac tccccgggtg ctatgggggtc ccggtgtacc	3000
taaagggaga aggaccccat gtgctagcca gaaatatact gtctcttgaa ggaaagcagg	3060
agctcagact cttagagcca gctgtggctt cggaaccaag gcctgacctg ggctgctatc	3120
ctaataattgg aggaggggccc tctcttccaa gccccaccct aagggttagc ccttgacaaa	3180
atcttggtgc gtctaggccc agccaggctt ttctgactaa ataagcaata agaggctcta	3240
agctgactga gttgcaagga ccttttccgc cctcccttgg atctccatgt ttctccagat	3300
ggcgggaagag catgtgccac cccctttcct aacagacttg tccaagtgtc tggcgtggga	3360
cccatgacca aagcccagga tggtctgggtg ggagtgtccc tgctgcatct gcatgaagcc	3420
cctgcttttt aggccctact cccatcagaa cctgcctgc ccacctgcaa cccccccca	3480
acaatgccat tcccacttgc ccagagaag ctactcggcc aaacctagcc agggctctgtt	3540
cttggtggacc agagccagcc tagtcattat ttgctgtcgg gtttccagtt tcaccgtgtg	3600
ttaggggtgag ggatgattgt aaaatttgct cctcaaagga atcaggccag actcaatttt	3660
gggagggcaa gacagggagg aggcgcttc atcccagact ctcttctagg gcttcccacc	3720
atcagccct cccacttgag actggtcttt gggaggcaat aggcaccat gcctggtcag	3780
caccaattca agccatgcca ggaatctgcc tacctgccag gttcagttct ttttaagggtc	3840
ctcttcaggg acacagtgtg tctctctgat tgggcttcta aatcaaaagc ctgatgttcg	3900
tgtccctctc atagggggag ctttggacac aggaccagtt tggaaaaggg tcaggtaagg	3960
gtttccactc tgcacattgt agagggaaca ctctgtaggc ccatgggtcc cttactagag	4020
aggttgagtg aatttgccct cagttaacat gggaccttct gtttagcttc ctcttgcttc	4080
ccaaagattt taagcatttt gtaaattgat aaactcacct ctggtaacag tggcccagac	4140

```

gctgctttgt gctaaaagca tgggaaatgt aaaggcagtc tttctctggg aaatggatgc 4200
tattctattc tgctgcccct acctgttcct gaggcctcat ttāgaaagaa aatccccctca 4260
gaaggctgtc tggcaccacag tgtcctagcc aggccaagta tatgagaaaag gtaagtccat 4320
tttccccttc aggtcctcag tggattactt aaccactgct gtccctcggg ccctttttcc 4380
taaacggggt tagttctgtc ttttttctcc ttttttctaa atgctggtaa atatttacat 4440
tcagccaggg aagaggaggc cagaggtcgg gccagctgcc ccattctttt aacgttgtag 4500
ggcctgcca tggagcggac cctcctcttt gggcctcgtg agcttttttg cttatcatgt 4560
tccatttcgt gccgctttcc cccttcaaga tgccatttgg agggtagggg atctgcttcc 4620
cactgtgact gggctatggg attctgacta ctttgcttac agattcatgg tttgataaat 4680
ttgttgattt ccaaaaacttg aaatgcagga cgccattaag tgtctgttta ttttttggga 4740
atatttgat tacttacaat taattaataa aagtgggttt aaaaaacctt tccaggaaaa 4800
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa 4833

```

```

<210> 15
<211> 859
<212> DNA
<213> Homo sapiens

```

```

<400> 15
atggacggcc cggccatcat caccaggtg accaacccca aggaggacga gggccgggtg 60
ccgggcgcgg gcgagaaagc ctcccagtg aacgtcagct taaagaagca gaggagccgc 120
agcatcctta gctccttctt ctgctgcttc cgtgattaca atgtggaggc ccctccaccc 180
agcagcccca gtgtgcttcc gccactggtg gaggagaatg gtgggcttca gaagccacca 240
gctaagtacc ttcttccaga ggtgacggtg cttgactatg gaaagaaatg tgtggtcatt 300
gatttagatg aaacattggg gcacagttcg tttaagccta ttagtaatgc tgattttatt 360
gttcgggttg aaatcgatgg aactatacat cagggtgatg tgctgaagcg gccacatgtg 420
gacgagttcc tccagaggat ggggcagctt tttgaatgtg tgctctttac tgccagcttg 480
gccaaagtat cagaccctgt ggctgacctc ctagaccgct ggggtgtgtt ccgggcccgg 540
ctcttcagag aatcatgtgt ttttcacgtt gggaactacg tgaaggacct gagtcgcctt 600
gggcgggagc tgagcaaagt gatcattgtt gacaattccc ctgcctcata catcttccat 660
cctgagaatg cagtgcctgt gcagtcctgg ttcgatgaca tgacggacac ggagctgctg 720
gacctcatcc ccttctttga gggcctgagc cgggaggacg acgtgtacag catgctgcac 780
agactctgca ataggtagcc ctggcctctg cctgcctccc gcctgtgcac tctggaacct 840
ctggcctcag gggacctgc 859

```

<210> 16
 <211> 754
 <212> DNA
 <213> Homo sapiens

<400> 16
 atgatgggga ggccctgcct gctcacagcg gggcgccct gcttgtggag gagaatggcg 60
 ccatccctaa ggcagacccc agtccaatac ctgctccctg aggccaaggc ccaggactca 120
 gacaagatct gcgtgggtcat cgacctggac gagaccctgg tgcacagctc cttcaagcca 180
 gtgaacaacg cggacttcat catcctgtg gagattgatg ggggtgggtcca ccagggtctac 240
 gtgttgaagc gtccctcacgt ggatgagttc ctgcagcgaa tgggcgagct ctttgaatgt 300
 gtgctgttca ctgctagcct cgccaagtac gcagaccag tagctgacct gctggacaaa 360
 tggggggcct tccgggcccg gctgtttcga gagtccctgcg tcttccaccg ggggaactac 420
 gtgaaggacc tgagccggtt ggggtcgagac ctgcggcggg tgctcactct ggacaattca 480
 cctgcctcct atgtcttcca tccagacaat gctgtaccgg tggcctcgtg gtttgacaac 540
 atgagtgaca cagagctcca cgacctctc cccttcttcg agcaactcag ccgtgtggac 600
 gacgtgtact cagtgtctcag gcagccacgg ccaggagct agtgaggggtg atggggccag 660
 gacctgcccc tgaccaatga taccacacc tctcccagg aagactgcc aggcctttgt 720
 taggaaaacc catgggcccgc cgccacactc agtg 754

<210> 17
 <211> 27
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic construct: polymerase binding site

<400> 17
 gaattaatac gactcactat agggaga 27

<210> 18
 <211> 25
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 18
 atgggcgaac tatacgagtg cgttc 25

<210> 19
 <211> 25
 <212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 19

atcaacgaca acttcgagat cgtcg

25

<210> 20

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 20

atgtcgctct tgcaaaaact aagc

24

<210> 21

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 21

tgaagatcct caccgagcgc ggcta

25

<210> 22

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 22

cagctggtgc gggagtacgg cttcc

25

<210> 23

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Primer.

<400> 23

gagctgtcgt tgagctttgg cg

22

<210> 24

<211> 23

<212> DNA

<213> Artificial sequence

<220>
 <223> Primer

 <400> 24
 actgggccta ttactactgg ctc 23

 <210> 25
 <211> 25
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 25
 caacgaagcc gagcgagcca tccag 25

 <210> 26
 <211> 26
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 26
 gcaacaactg ggccaagggt cattac 26

 <210> 27
 <211> 26
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 27
 gccttccaag agcacgacgt acaaag 26

 <210> 28
 <211> 26
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 28
 ctgcgccaatc aagtaccttg tgctgc 26

 <210> 29
 <211> 25
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

 <400> 29
 cttcgctcgc acctcagaaa cgatc 25

 <210> 30
 <211> 25
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 30
 caacggaact aacggccgct ccgag 25

 <210> 31
 <211> 22
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 31
 ctcgccattg ttctcctggt gg 22

 <210> 32
 <211> 25
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 32
 cttgtctgct gctggttcaa catgg 25

 <210> 33
 <211> 25
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Primer

 <400> 33
 gcggttgag tagccaaact cgttg 25

 <210> 34
 <211> 25
 <212> DNA
 <213> Artificial sequence

 <220>

<223> Primer

<400> 34

ttataggata tcttcgattt tcggc

25

<210> 35

<211> 26

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 35

gaccggactc gtcatactcc tgcttg

26

<210> 36

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 36

tgcgagctc gcccatgtag acctg

25

<210> 37

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 37

cgcgtggatt ggggaagaag gtc

23

<210> 38

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 38

ccgtaaaacc gcgcgatta aagt

24

<210> 39

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 39
tgggtcatggt cacgaatccg aatc

24

<210> 40
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 40
cttggcatcg aacatctgct gggtcag

27

<210> 41
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 41
cgatcagaag tggatcgcg tcctta

26

<210> 42
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 42
ccctggctga agcagaactt catg

24

<210> 43
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 43
tatggcataa aaggtgtggc cattc

25

<210> 44
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 44
gttctcgcca tcgttgagat ctgc

24

<210> 45
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 45
cgtacatgag gtagaccctg ga

22

<210> 46
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 46
cgcccgatc tactcagatc agcaagg

27

<210> 47
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 47
tccaccaccc tgtgttgctg ta

22

<210> 48
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 48
catctctgat ctgactgct ccagcag

27

<210> 49
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 49

tgccctcacc caaggtctct gacactgtgg

30

<210> 50
 <211> 30
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 50
 ctgtggccat ggagggaaac agtggcttcc

30

<210> 51
 <211> 28
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 51
 gcaaccgcag gcacgactgt ttacggag

28

<210> 52
 <211> 29
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 52
 ccacgcctg cgaaacctcc ccaggtaga

29

<210> 53
 <211> 28
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 53
 gcagtgaaca gcacacattc aaagagct

28

<210> 54
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 54
 accacagtcc atgccatcac

20

<210> 55
 <211> 27
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 55
 gggtcagaga gtggtgatgc cacagtg

27

<210> 56
 <211> 28
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 56
 cttgaacagc tcctggatgg cagtgctg

28

<210> 57
 <211> 28
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 57
 agaagtccag gagcagctga gggagcac

28

<210> 58
 <211> 30
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 58
 agatgaccat ccggaagaag ttggccttgt

30

<210> 59
 <211> 30
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 59
 agccaactca gctggactct ctccagcttc

30

<210> 60
 <211> 30
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 60
 tgcggtttat attatcctgc acgccgggag

30

<210> 61
 <211> 27
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 61
 ggagccctat gcagggtaag ggaataa

27

<210> 62
 <211> 28
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 62
 aactatttct gggtcactcc ttagacac

28

<210> 63
 <211> 30
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 63
 ctggataagt tactgaagag tgggcttttg

30

<210> 64
 <211> 29
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer

<400> 64
 caccggttcg agtccccgga gaggatatc

29

<210> 65
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 65
gggctttgat ttttggagcc accttggtg

28

<210> 66
<211> 29
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 66
gctgggagga atgctttcta atgcatttg

29

<210> 67
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 67
cagacgacaa gttacatgca acatg

25